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THE iDINO PROJECT SPECIAL REPORT

- *ORIGINAL BIOMATERIALS IN FOSSILS*
- *SOFT BONE MATERIAL FROM A BROW HORN OF A TRICERATOPS*
- *DINOSAUR TISSUE OR BACTERIAL BIOFILMS?*
- *DINOSAUR PEPTIDE PRESERVATION AND DEGRADATION*
- *HELL CREEK FORMATION: LAST GASP OF THE PRE-FLOOD DINOSAURS*
- *RADIOCARBON IN DINOSAUR AND OTHER FOSSILS*

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Haec Credimus

For in six days the Lord made heaven and earth, the sea, and all that in them is, and rested on the seventh.—Exodus 20:11

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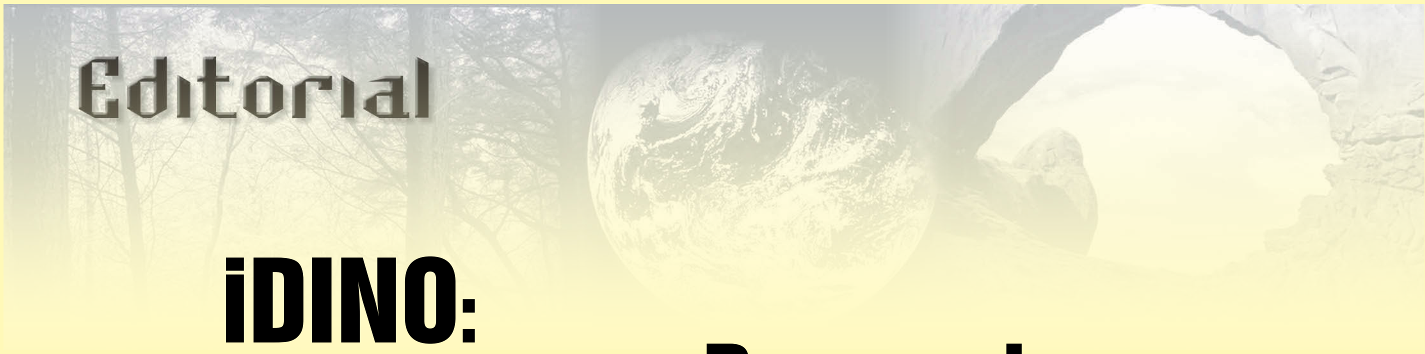


iDINO: An Important Research Project Supported by CRS Member Donations

The purpose of the Creation Research Society is to promote research in support of the creation model. It is my pleasure to present this special issue of the *Quarterly* dedicated to perhaps the most significant research ever sponsored by the society, the study of the preservation of soft tissue in dinosaur fossils. It was more than 20 years ago that Mary Schweitzer discovered biomolecules in dinosaur bones, but it was not until 2005 that she found soft tissue in a dinosaur bone. To say that Schweitzer's work has been controversial would be an understatement. No one thought it possible that such unfossilized tissue could remain preserved in rock for more than 60 million years. The scientific world begrudgingly has come to accept Schweitzer's work, and there has been much discussion on how tissue could remain intact for such a long time. One obvious solution to this dilemma is that the fossils in question were buried just thousands of years ago, not millions, as the creation model requires. Therefore, it is appropriate that creationists take the lead in the study of soft tissue in fossils.

The centerpiece of this research is the iDINO (Investigation of Dinosaur Intact Natural Osteo-tissue) project, funded by the Creation Research Society. In this issue Kevin Anderson, project leader (and director of the Society's Van Andel Creation Research Center), gives an overview of the iDINO project. In a separate article he addresses arguments that the dinosaur tissue is merely bacterial biofilm. Mark Armitage discusses the collection and analysis of soft tissue in a triceratops horn. Brian Thomas, of the Institute for Creation Research, has two articles, one an overview of the history and significance of original biomaterial in fossils, and the other on radiocarbon in fossils (coauthored with Vance Nelson). John DeMassa and Edward Boudreaux discuss the preservation and degradation of dinosaur peptides. Finally, Timothy Clarey, of the Institute for Creation Research, discusses the geology of Hell Creek, Montana, where many of the fossils containing unfossilized tissue have been found (including the triceratops horn). I wish to thank the work of all of these authors but especially Kevin Anderson, who planned the articles and commissioned the authors.

I also want to thank the members of the Society who donated to the iDINO project so that the original research of on-site data collection and analysis was possible. The iDINO project is not finished. There is more work to be done. The second phase will focus more specifically on the fossilization process. We have also begun preliminary filming for an iDINO video. I encourage members to contribute to this worthwhile project so that further work can continue.

> This is perhaps the most significant research ever sponsored by the society—the study of the preservation of soft tissue in dinosaur fossils.

Danny R. Faulkner, Ph.D.
Editor
Creation Research Society Quarterly

iDINO

Investigation of Dinosaur Intact Natural Osteo-tissue

A CRS Research Initiative

The iDINO Project

Kevin Anderson, Ph.D.
iDINO Project Leader
Van Andel Creation Research Center
Chino Valley, AZ

Dinosaurs have long held a unique fascination for people. Speak to a group of elementary-age children, and you quickly learn that many of them can identify the common names for a number of the larger dinosaurs. Evolutionists have recognized the value of using this enthusiasm to effectively indoctrinate the public about evolution (e.g., the January 2015 issue of *Smithsonian* uses dinosaurs as part of their "evolution world tour" segment). Questions, such as how Noah could fit dinosaurs on the ark or why dinosaur fossils are not found mixed with human fossils, are often naively used to contradict both biblical and scientific arguments for a recent creation.

The Creation Research Society began its iDINO research initiative with the expressed purpose of addressing questions about dinosaurs from a creationist perspective. The primary focus of the project is to study soft tissue in dinosaur fossils (and subsequently other so-called ancient fossils). As explained by Thomas (2015), intact tissue, cells, and biomolecules have been reported in dinosaur fossils for several decades. Their presence presents a significant challenge to the assigned date of these fossils, which thus challenges the current evolutionary-biased standard dating methods.

Can tissue (aggregates of interconnected cells) retain its natural, flexible characteristics in so-called ancient fossils? Can cells within this tissue retain their structural integrity and morphology? Can biomolecules (such as protein and DNA) actually survive 60 million years inside a fossilized and buried bone? What is the natural process that enables such preservation?

> CRS began its iDINO research initiative with the expressed purpose of addressing questions about dinosaurs from a creationist perspective. The primary focus of the project is to study soft tissue in dinosaur fossils (and subsequently other so-called ancient fossils).

Tissue containing fossils are not preserved in permafrost. In fact, fossils in Montana's Hell Creek Formation are potentially subjected to wide temperature fluctuations, which is certainly not conducive to biomolecule preservation. The *Triceratops* horn that was analyzed as part of the iDINO project (Armitage and Anderson, 2013; Armitage, 2015) was buried under no more than 30 cm of sandstone (from the top surface) with the base partially exposed. Such depth provides little thermal protection for the specimen.

> Regardless of any other environmental conditions that would affect tissue preservation, environmental radiation will always be a significant factor. For the tissue to be original, the fossils cannot be millions of years old.

While Dr. Mary Schweitzer's work was not the first to observe preserved tissue/cells in dinosaur fossils, her work drew widespread attention to their existence (Schweitzer et al., 2005, 2007). Understandably, many of her evolutionist peers recognized the possible consequences of her findings and reacted to protect the evolution model's need for long ages—regardless of the data. She acknowledged that one reviewer told her,

> He didn't care what the data said; he knew that what I was finding wasn't possible. I wrote back and said, "Well, what data would convince you?" And he said, "None." (Yeoman, 2006)

Geochemist, Jeffrey Bada, insists that tissue/cells could not possibly survive millions of years.

> He says the cellular material Schweitzer found must be contamination from outside sources. Even if the *T. rex* had died in a colder, drier climate than Hell Creek, environmental radiation would have degraded its body, Bada says: "Bones absorb uranium and thorium like crazy. You've got an internal dose that will wipe out biomolecules." (Yeoman, 2006)

Thus, since we "know" these fossils are 60, 70, or 80 million years of age, the environmental radiation would destroy all original tissue and biomolecules. Whatever Dr. Schweitzer's group is detecting, Dr. Bada would conclude it cannot possibly be original dinosaur tissue. Yet, biostatistician Martin McIntosh admits that he "cannot right now make a plausible argument" that it is not original dinosaur tissue, and he acknowledges that "the door is closing on plausible alternatives" (Service, 2009, p. 578).

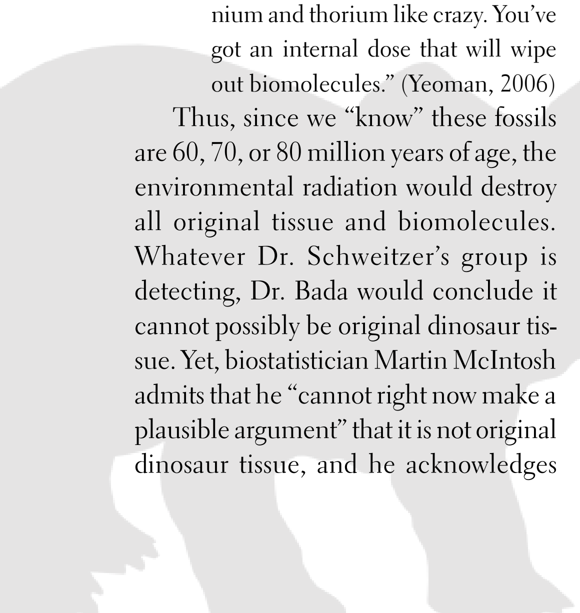
Hence, this becomes the dilemma for paleontologists. Dr. Bada is factually correct. Regardless of any other environmental conditions that would affect tissue preservation, environmental radiation will always be a significant factor. For the tissue to be original, the fossils cannot be millions of years old. So, is the tissue original?

Dr. Schweitzer and her team detected pliable forms of tissue containing morphologically detailed cells (Schweitzer et al., 2005, 2009, 2013). Armitage and Anderson (2013) also observed similar tissue with morphologically detailed cells. Is the detection of such cellular detail a microscopic error? Is such pliable tissue the result of a biofilm contamination (Anderson, 2015) or other forms of contamination?

Asara et al. (2007) detected collagen amino acid sequences from a *T. rex* fossil dated at 68 million years. Working independently, Bern et al. (2009) analytically confirmed the presence of avian-type proteins in the same *T. rex* fossils. However, instead of suggesting these were original *T. rex* proteins, Bern and coworkers offer alternative explanations. These alternatives include suggestions that cosmetics accidently contaminated the samples, traces of ostrich bone proteins lingered in the mass spectrometer, or "a bird died on top of the *T. rex* excavation" site (Bern et al., 2009, p. 4331). Are they suggesting all dinosaur specimens have been contaminated with cosmetics or that all the mass spectrometers retained traces of avian samples? Does collagen detected in fossils of *Brachylophosaurus* (Schweitzer, et al. 2009) suggest a bird also died on this excavation site?

The extremity of these suggestions (even willingness to assume poor lab technique) demonstrates that the evolution paradigm provides fertile grounds to eagerly consider virtually any idea other than dinosaur fossils are "young." In other words, there *has* to be alternative explanations. If these alternatives help support standard evolutionary teaching, many will willingly consider them, no matter how silly or embarrassing.

Creationists would argue that a far more scientifically consistent interpretation is that these fossils are simply a few thousand years old. In fact, even evolutionists see the logical consistency of this interpretation. Dr. Schweitzer admits that when she first discovered the soft-tissue, her graduate advisor, professor Jack Horner, warned that "the creationists are just going to love you" (Ruppel, 2014). Yet, Schweitzer is very dismissive of creationists using the soft-tissue data. She laments that creationists have a "misunderstanding of what is



science" and are "trying to rewrite the rules of science" (Healy, 2009). She has expressed annoyance "that young earth creationists take my research and use it for their own message, and they are misleading people about it" (Ruppel, 2014).

Apparently, when creationists use any data to support their position, it is rewriting "the rules." These "rules" presumably are that all data must be viewed within an evolutionary context. Since many evolutionists see the logical consistency that this soft-tissue indicates a younger age for the fossils (hence, the resistance that Dr. Schweitzer has encountered regarding her findings), her dismissal of the creationists' view is both emotional and illogical. She even admits that "it's not surprising" that creationists have drawn these conclusions (Ruppel, 2014). Nor should it be surprising. Such conclusions are obvious—soft tissue and biomolecules degrade long before a fossil becomes 65 million years of age (Thomas, 2015; Demassa and Boudreaux, 2015).

Jack Horner notes that when he first met Mary Schweitzer, she was a young-earth creationist (Horner and Gorman, 2009). It would appear she was not very educated in the science of creation and thus rather easily swayed by evolutionary teachings once she entered college. Regrettably, this is a rather common situation. Lack of adequate understanding of both the biblical and scientific foundation for a recent and direct creation makes young people very vulnerable to the indoctrinations they receive in their college classes. Once indoctrinated, as was the situation for Dr. Schweitzer, the individual frequently becomes rather belligerent to any creationist teaching or argument. Perhaps this is because they now feel embarrassed they had once accepted creation, or because they feel compelled to correct those ignorant enough to have not "seen the light" as they have now.

What is clear is that their early understanding of creation science (and the Bible?) was incomplete and frequently rather naive. Their understanding may have been based upon some generalized or overly simplistic book (of which there are far too many). The consequence though, is the assumption that they understood creation. Thus, when they failed to answer questions raised in college classes, they mistakenly assumed that the questions were unanswerable. They also assumed that the answers given by the professors were not flawed as well (a rather erroneous assumption). Hence, they concluded that evolution and great ages for the earth are scientifically verified, and feeble little creation science is nothing more than a few superficial ideas and numerous misnomers from misguided Bible thumpers.

With this mindset, these "former creationists" now assume they have a full understanding of the creation model and thus have no reason for further study. As a consequence, they typically are blind to any argument for creation or even any criticism of evolution. This blindness pushes them forward in their evolutionary thinking, ignoring obvious biblical and scientific contradictions.

Self-described "former creationist" Denis Lamoureux claims to accept biblical teachings yet argues that the Bible "makes statements about how God created living organisms that in fact never happened" (Lamoureux, 2013, p. 56). He further states that "real history in the Bible begins roughly around Genesis 12" (Lamoureux, 2013, p. 44) and admits that he takes "a very counterintuitive way to read scripture" (Lamoureux, 2013, p. 63). Dr. Laumoureux is forced to these conclusions as a means of reconciling obvious biblical inconsistencies with evolution (e.g., creation in six solar days, Adam and Eve as the first humans, the creation of plants before the sun, etc.).

Such an unfortunate perspective undermines any and all biblical authority. If neither Adam nor Noah (Gen. 1, 7) really existed, how does Dr. Lamoureux know that Abraham or Jacob existed? What is his basis for this distinction? How does he know if Jesus' instantaneous calming of the sea or even His resurrection also "never happened"? After all, both these supernatural events clearly contradict contemporary scientific knowledge: Stormy seas cannot instantaneously become calm, and dead bodies cannot suddenly come back to life. Yet, this is the very "science" he uses to justify that the Bible makes statements about creation that never happened.

So enamored with the Darwinian doctrine he was taught in college, Karl Giberson reflects upon how his "fundamentalism" was eroded by the "acid" of evolution (Giberson, 2008). He admits this "acid dissolved Adam and Eve; it ate through the Garden of Eden; it destroyed the historicity of the events of creation week" (Giberson, 2008. p. 10). Dr. Giberson poses many questions about the historical place for Adam and Eve and how to reinterpret the creation week. However, he offers neither clear answers nor biblically consistent thoughts on these matters. His so-called "scientific knowledge" simply forces him to reject biblical teachings that do not readily fit within his evolutionary worldview. The shreds of the biblical record that remain after his "acid test" are somehow still supposed to be the viable and authoritative Word of God.

Unwavering allegiance to Darwinism also requires straining the biblical doctrines of sin and the Fall. This is exemplified by a Biologos essay that attempts to explain the origin of sin within the context of human evolution. Rather than being the first humans to sin (Romans 5:12–14; 1 Timothy 2:14), the biblical Adam and Eve are relegated to a role of "representing 'everyperson'—that is, each one of us" (Davis and Collins, 2014). Eve, the "mother of all" humans (Genesis 3:20) is apparently not the mother of anyone. The spiritual separation resulting from "the fall," as explained in Genesis 3:13–19 and Romans

If the soft tissue is original, then a very plausible interpretation is that these dinosaur fossils are actually young.

5:12–19, does not start with the single action of Adam. Rather, this separation is reduced to a "sedimentation of thousands of years of human choices of evil" (Davis and Collins, 2014). Readers can draw their own conclusions about the biblical impact this interpretation has on sin and redemption.

If biblical creationists state a position that evolution is not consistent with the Bible, it is because of failed attempts, such as those of Dr. Lamoureux and Biologos, to reconcile the two positions. This failure clearly illustrates the conflict. As Dr. Lamoureux admits, it takes a "counterintuitive" interpretation of the Bible to arrive at his position. Why? Because what the Bible clearly teaches (e.g., six-day creation, creation of Adam and Eve, creation of the earth before the sun, etc.), simply will not fit into his paradigm. Why does he hold to this paradigm, despite its biblical conflicts? Good question.

During the twentieth century, evolutionists were successful at equating evolution with science. This interchange of words has been used to twist the conflict into "creation versus science" rather than "creation versus evolution." By this mere rewording, creation is then cast as an opposing outsider of science. Since science is viewed as an objective analysis and understanding of information, creation (and consequently the Bible?) is automatically marginalized as a subjective "nonscience" and effectively reduced to virtual irrelevance. Simply by playing such word games, the debate is considered over. Apparently, for many, such as Dr. Lamoureux, this means the Bible must either bend to the demands of evolution (i.e., his version of science) or break in the process.

How, then, are we to view this perception? First, it is important to recognize that evolution is *not* equivalent to science. Science is a tool. Evolution is a perspective. At best, evolution is a human interpretation of data obtained from scientific methods. This is clearly not a situation of identifying evolution as being the same as science. Methods of experimental testing are not the same as the human interpretation of the test results. It is not "creation versus science"; rather, it is creation versus human opinion (and all the bias and emotion that entails). Word games to interchange evolution and science do nothing to clarify the boundaries of each. Rather, such word games appear to be intended to confuse, giving evolution a legitimacy it does not deserve and an authority it has never earned.

This returns us to the original question regarding proper interpretation of scientific data. In contemporary studies of science, evolution is simply assumed to be true. This assumption is then used to interpret all experimental results and data (Anderson, 2012). Assigning ages in the millions of years to fossils is less an empirical conclusion than it is a presupposition. Evolution requires vast geologic ages. Thus, geologists are trained to use this presupposition of geologic ages as a template rather than an idea to test or demonstrate (Reed, 2013). Rocks and fossils are interpreted based upon this template, and then the interpretation is declared a proof for evolution. The circularity is evident and has been repeatedly challenged by creationists.

In fact, not only is the presupposition of evolution the standard for all data interpretation, but it must be a specific version of neo-Darwinism. Just accepting materialistic evolution is not enough. Even several prominent evolutionists recently complained that they are having difficulty publishing ideas that are not fully in line with standard neo-Darwinian teaching (Laland et al., 2014). They lament that their views of nonrandom variation evoke "an emotional, even hostile, reaction among evolutionary biologists" (Laland et al., 2014, p. 162). Creationists would suggest that this all sounds very familiar. Interpretations that deviate too far from the standard evolution paradigm are simply not acceptable.

How should preserved "biomolecule-containing soft tissue" in dinosaur fossils be interpreted? First, it is important to ignore the presupposition of ancient ages. This presupposition offers no scientific insight and serves no productive purpose. Removing any unnecessary presuppositions now frees us to consider the most consistent and viable interpretation, regardless of whether it aligns with specific popular worldviews. Follow the data where it takes us. Is not that supposed to be the objective of scientific inquiry?

Therefore, discovery of this preserved tissue has three possible general interpretations: (1) the material is original tissue that was preserved for millions of years by some physical process; (2) the material is original tissue that required no special preservation because the fossil is only a few thousand years of age; or (3) the material is not original tissue but is the product of a microbial biofilm or other contaminating processes that mimic biomolecule-containing tissue.

If the soft tissue is original, then a very plausible interpretation is that these dinosaur fossils are actually young (i.e., no more than a few thousand years of age). This younger age contradicts the standard dating methodology used to originally establish the fossils' ages as more than 65 million years. Such contradiction draws the entire geological dating paradigm into question and further exposes the error of accepting evolution as a presupposition for the dating paradigm.

The following articles in this special iDINO issue will seek to address the adequacy of each of these possible interpretations. While none of these articles are offered as a "final word" on these topics, they are intended to provide a detailed and reasoned analysis of each interpretation. Readers can judge for themselves which interpretation is the more consistent and valid, but I challenge that when evolutionary presuppositions are ignored, a recent creation stands as a very strong and viable interpretation of the soft-tissue data.

References

Anderson, K. 2012. There can be only one. *Creation Research Society Quarterly* 49:72–77.

Anderson, K. 2015. Dinosaur tissue or bacterial biofilms? *Creation Research Society Quarterly* 51:259–267.

Armitage, M. 2015. Soft bone material from a brow horn of a *Triceratops horridus* from Hell Creek Formation, MT. *Creation Research Society Quarterly* 51:248–258.

Armitage, M., and K.L. Anderson. 2013. Soft tissue of fibrillar bone from a fossil of the supraorbital horn of the dinosaur *Triceratops horridus*. *Acta Histochemica* 115:603–608.

Asara, J.M., M.H. Schweitzer, L.M. Freimark, M. Phillips, and L.C. Cantley. 2007. Protein sequences from mastodon and *Tyrannosaurus rex* revealed by mass spectrometry. *Science* 316:280–285.

Bern, M., B.S. Phinney, and D. Goldberg. 2009. Reanalysis of *Tyrannosaurus rex* mass spectra. *Journal of Proteome Research* 8:4328–4332.

Davis, T., and R. Collins. 2014. Evolution and original sin by Robin Collins, Part 2: the historical ideal view of Adam & Eve. The Biologos Forum. http://biologos.org/blog/evolution-and-original-sin-by-robin-collins-part-2-the-historical-ideal-vie (Accessed December 2, 2014).

Demassa, J.M., and E. Boudreaux. 2015. Dinosaur peptides: preservation and degradation. *Creation Research Society Quarterly* 51:268–285.

Giberson, K.W. 2008. *Saving Darwin*. HarperCollins Publishers, New York, NY.

Healy, D. 2009. Paleontologists take issue with creation-based museum. *The Billings Gazette*. http://billingsgazette.com/news/features/magazine/article_ffd7630c-baaf-11de-84f0–001cc4c002e0.html (Accessed June 3, 2012).

Horner, J., and J. Gorman. 2009. *How to Build a Dinosaur: Extinction Doesn't Have to Be Forever*. Penguin Group, New York, NY.

Laland, K., T. Uller, M. Feldman, K. Sterelny, G.B. Müller, A. Moczek, E. Jablonka, J. Odling-Smee, G.A. Wray, H.E. Hoekstra, D.J. Futuyma, R.E. Lenski, T.F.C. Mackey, D. Schluter, and J.E. Strassmann. 2014. Does evolutionary theory need a rethink? *Nature* 514:161–164.

Lamoureux, D. 2013. No historical Adam: evolutionary creation view. In Barrett, M., and A.B. Caneday (editors), *The Historical Adam*, pp. 37–65. Zondervan Publishing, Grand Rapids, MI.

Reed. J.K. 2013. *Rocks Aren't Clocks*. Creation Book Publisher, Powder Springs, GA.

Ruppel, E. 2014. Not so dry bones: an interview with Mary Schweitzer. *The BioLogos Forum*, posted July 21, 2014. http://biologos.org/blog/not-so-dry-bones-an-interview-with-mary-schweitzer (accessed September 12, 2014).

Service, R.F. 2009. 'Protein' in 80-million-year-old fossil bolsters controversial *T. rex* claim. *Science* 324:578.

Schweitzer, M.H., J.L. Wittmeyer, J.R. Horner, and J.K. Toporski. 2005. Soft-tissue vessels and cellular preservation in *Tyrannosaurus rex*. *Science* 307:1952–1955.

Schweitzer, M.H., J.L. Wittmeyer, and J.R. Horner. 2007. Soft tissue and cellular preservation in vertebrate skeletal elements from the Cretaceous to the present. *Proceedings of the Royal Society B* 274:183–197.

Schweitzer, M.H., W. Zheng, C.L. Organ, R. Avci, Z. Suo, L.M. Freimark, V.S. Lebleu, M.B. Duncan, M.G. Vander Heiden, J.M. Neveu, W.S. Lane, J.S. Cottrell, J.R. Horner, L.C. Cantley, R. Kalluri, and J.M. Asara. 2009. Biomolecular characterization of protein sequences of the campanian hadrosaur *B. canadensis*. *Science* 324:626–631.

Schweitzer, M.H., W. Zheng, T.P. Cleland, and M. Bern. 2013. Molecular analyses of dinosaur osteocytes support the presence of endogenous molecules. *Bone* 52:414–423.

Thomas, B. 2015. Original biomaterials in fossils. *Creation Research Society Quarterly* 51:234–247.

Yeoman, B. 2006. Schweitzer's dangerous discovery. *Discovery Magazine*. http://discovermagazine.com/2006/apr/dinosaur-dna/article_view?b_start:int=1&-C= (accessed June 3, 2012).

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Original Biomaterials in Fossils

Brian Thomas*

Abstract

Characterizing and understanding original tissue fossils holds great interest because of what these fossils imply about the incongruous timelines that help define biblical creation versus evolution. General expectations suggest, and repeatable lab experiments demonstrate, that biomaterials original to fossilized organisms have a finite shelf life. Tissues in fossils confront evolutionary time. This article reviews generations of research that have exposed and explored original biomaterial fossils, establishing beyond reasonable doubt the reality of endogenous fossil tissues as a genuine feature of Earth layers. It also surveys original biomaterial fossils in geological context and updates both the secular and creation-based research status of this science. We conclude that original biomaterials found throughout the fossil record confirm the biblical timeline of Creation-Flood geology.

Introduction

The broadest definition of a fossil as the remains of a once-living organism ranges from a partly rotten carcass to a solid, mineralized body part. However, even the typical professional scientist seems to understand fossils almost strictly in terms of hard body parts or rare impression fossils such as prints or casts found as solid rock. Supposedly, shells and teeth preserve most readily, and bones preserve if replaced by minerals in a process called secondary mineralization. This common and widespread understanding leaves little room to expect fossils to still retain original bone mineral bioapatite, let alone other, softer body tissues to have persisted without having been mineralized. And yet such soft parts, or at least their partially decayed remnants, have been described in technical literature for decades.

First, however, clarifying certain terms clears verbal clutter that might otherwise cloud this discussion. The phrase “soft tissue” carries ambiguity, since it is found in secular literature descriptions of mere impressions, like casts or molds in solid rock matrix—even though these may have no organics endogenous to the organism that left its imprint. For example, a dinosaur footprint preserved the “soft tissue” outline of a dinosaur’s foot, but none of its original biochemistry. These “soft tissue” impressions are of less interest to the question of evolutionary time, since the minerals of which rocks are composed are much more resistant to chemical decomposition than bodily organics.

For this reason, we propose using the phrase “original biomaterials” to refer to the many instances of virtually unaltered organic residue found in fossils. By “original” is meant that the organic material described from within the fossil came from the organism that occurs as a fossil, and did not somehow originate from an outside, perhaps more modern source that then carried the organics into the fossil. In other

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words, "original" opposes "contaminant." "Biomaterial" seems broad enough to convey all the range of fossil organics described in paleontological literature, from the tiny vitaminlike chlorophyll molecules that pervade shale and were original to algae, to whole or nearly whole mastodon carcasses found too deeply buried in permafrost for even wild, starving dogs to access its ancient flesh. There is somewhere in the literature an example of probably every point in the fossil biomaterial continuum that ranges from an entire large animal to the last partly intact biochemical from long-buried animals or plant. Of greatest interest to origins discussions are those original biomaterial fossils with the highest integrity and which are found in the lowest rock layers. Indeed, rock types from all geologic "eras" contain original tissue fossils.

Why does this investigation matter? In brief, the outcomes can help adjudicate between two timelines and the two paradigms that they represent. Evolutionary time posits that billions of years, while biblical creation maintains that only thousands of years, have elapsed since the formation of fossiliferous Earth materials. If original biomaterials can be shown to last for hundreds of millions of years, then their discovery in the field does not discern between these two paradigms. In that case, either perspective could accommodate the data. But if original biomaterials can be shown to last no more than hundreds of thousands of years, then their many discoveries and descriptions exclude evolutionary time while confirming the Bible's timeline. For this reason, the first section of this paper identifies studies on the decay rates of two biomaterials that have been reported in several fossils. If repeatable science establishes that biomaterials could not last through deep time and that original biomaterials occur in fossils, then it opposes the now iconic evolutionary timeline, even as many other sciences already do.

Protein and DNA Decay Rates

Merely an intuitive sense of how short a time vertebrate body tissues should last, even ensconced in sedimentary rock, prompts puzzling questions from those of both creationary and evolutionary perspectives. Few anticipate that biochemicals and even whole tissues could last any longer than perhaps 1,000 years. On this basis, the evolutionist must answer the question of how original biomaterials could last for tens of millions of years. But again on this basis the biblical creationist must answer the question of how original biomaterials could have lasted since Noah's Flood, over four to five millennia. It would seem on the surface that this fossil evidence entangles both paradigms. However, research on biomolecular decay rates supplies objective data that erase this creationist question but sharpen the evolutionary dilemma.

Collagen Decay

Collagen occurs in all multicellular animals, and is the most abundant protein of vertebrates. Repeated experiments accelerate bone collagen decay under high temperature, obtaining a rate that can then be converted to a decay rate under reasonable earth surface temperatures, ultimately supplying a maximum expected lifetime for that molecule. At 90°C, collagen in bone decays almost completely after a month. Several bioanalytical techniques have been used to assess the integrity of collagen at points throughout the experiment. Each begins by dissolving bioapatite in weak acid buffer. The overwhelming prevalence of collagen in bone is intensified during fossilization because of its insolubility in water. Thus, demineralization of fossil bone can yield a high purity of collagen. In one technique, the collagen thus separated is first weighed, then thoroughly desiccated before reweighing. Subtracting the two mass values reveals the protein's tendency to lose water weight, a correlative to its integrity. As the collagen molecule disintegrates over time, the numbers of water molecules it can absorb decreases until no collagen structure remains and hence no water is retained.

Alternatively or in addition, bone collagen fractions collected from different decay times can be run on an SDS-PAGE gel that separates molecules by molecular weight. Except for a spike in the first few days of the typically one-month experiment, the longer collagen is held under 90°C, the more it degrades, and thus shortens. Shorter proteins have lower molecular weights and travel further through the SDS-PAGE gel pulled by the same voltage. After a month at 90°C, these gels reveal collagen extracts that have degraded to tiny peptides and individual amino acids, as confirmed by comparison with proteins of known molecular weights.

Resulting decay rates are then converted using a version of the Arrhenius equation into rates that are based on much lower temperatures (Thomas, 2013). The principle at work behind this conversion describes a degradative reaction based primarily on chemical kinetics, such that an increase in temperature also increases the occurrence of molecular collisions and thus the chemical reactions that break down biomaterials like collagen. However, at certain temperatures cross-linking occurs, producing a new and resistant material (see discussion below regarding kerogen). Accordingly, bone collagen held at a steady annual temperature of 10°C will completely disintegrate under the best possible preservation conditions after 300,000 to 900,000 years (Buckley et al., 2011). Expressed another way, Collins and colleagues have determined a half-life for bone collagen at 7.5°C of 130,000 years (Buckley et al., 2008). We can solve the standard logarithmic decay half-life equation for time (t) until collagen decays to 1%, at which point it is no longer detectable and in practice no longer recognizable as collagen.

$N = N_o e^{-\lambda t}$, Where N is the number of intact (or still-detectable) collagen molecules after time (t), N_o is the number of original collagen molecules, e is the base of natural logarithms, and λ is ln 2/half-life. Setting N to $0.01N_o$, $t = 8.64 \times 10^5$ years—fewer than one million years at 7.5C, the average temperature in Montana where the most famous dinosaur proteins were recovered (see below).

This experiment-based decay rate of collagen was determined by archaeologists who investigate materials they believe date from tens of thousands of years ago, and not paleontologists who work with material they have age-dated by biostratigraphy at tens or hundreds of millions of years. So when these archaeologists heard about original dinosaur protein, they of course rejected its veracity on the grounds that collagen could not have lasted since the Mesozoic. Thus, this illustrated how known science accounts for the persistence of bone collagen for thousands of years since the Flood, while challenging deep time.

DNA Decay

Michael Collins and colleagues used the experiment-based collagen decay rates described above to rebut Mary Schweitzer's 2007 *Science* paper describing *Tyrannosaurus rex* tissues, as discussed below (Buckley et al., 2008). Later, Collins worked with Allentoft to experimentally investigate DNA decay rates. This team conducted a rigorous investigation of 158 fossil birds to determine a half-life of 521 years for moa mitochondrial DNA in bone at 13.1°C, the average temperature of New Zealand where the samples were collected (Allentoft et al., 2012). Their results showed that DNA decays more slowly than prior experiments with DNA decay kinetics, and attributed the difference to pH. Preceding researchers experimented with DNA at pH 5, but the moa bone microenvironments were near pH 7. Whereas an increasingly acidic environment undoubtedly accelerates DNA decay, I suspect that their slower-than-expected fossil-DNA-based rate is instead, or in addition to pH considerations, a function of their method of time-calibration. Allentoft et al. (2012) used uniformitarian carbon dates for the bones from which they extracted the DNA, then tied their DNA decay curve to carbon years, assuming that carbon years equal calendar years. However, carbon years often fail to correlate to actual time, and in general, older carbon dates separate further from an artifact's actual age (Snelling, 2008).

Several indicators suggest that 521 years represents an unrealistically long half-life for DNA. For example, Theodore Siek cited forensics studies showing that DNA degradation in natural environments including domestic settings, in water, and in ice, imposes relatively narrow time restrictions on expected DNA recovery for forensics analyses (Siek, 2010). Nevertheless, for the sake of argument one can overlook the radiocarbon bias incorporated into the Allentoft DNA decay rate, since even this unrealistically large 521-year half-life refutes many claimed ages for DNA found in fossils.

DNA in Fossils

For example, the still-unsullied integrity of DNA from amber-encased insects dramatically confronts the evolutionary age assignments. A series of reports from the 1990's led by Raul Cano, then of California Polytechnic State University, confirmed DNA in amber insects. One study, published in *Nature*, asserted an age of between 120 and 135 million years for a weevil's DNA from Lebanese amber (Cano et al., 1993). This and similar results were disputed even before Allentoft's DNA half-life was published. Workers at The Natural History Museum in London tried to repeat the DNA extraction protocols from Dominican amber, but obtained negative results. This casts doubt on the original reports, but is complicated by the general expectation that "DNA is extremely fragile, degrades in water and tends to fall apart and lose its 'signature' very easily" (Austin, n.d.). So, a responsible secular investigator who wishes to report DNA from ancient fossils faces the challenge of offering some kind of solution to a seemingly impossible question: How could DNA have lasted millions of years? A much easier path would avoid this dilemma entirely by leaning toward the interpretation that no endogenous fossil DNA persists in samples that are too old to contain it. For example, some secular researchers claim that DNA from ancient ambers originated from recent bacterial contamination. However, they have not yet produced a comparison of bacterial, modern insect, and fossil insect DNA sequences to support this contention, leaving for its basis the sheer need to rescue millions-of-years ages. More work needs to be done to verify the many solid clues pointing to genuine ancient DNA—and it needs to happen soon, before that DNA completely decays.

Dinosaur DNA reports exist, but have not yet brought forth enough clarity to make an airtight case against millions-of-years age assignments. In 1994, Woodward and colleagues described DNA they captured from dinosaur bone (Woodward et al., 1994). Their report was quickly and roundly rebuffed by experts who claimed that the detection techniques used could not yet rule out contamination. Since then, ancient DNA (aDNA) recovery techniques have dramatically improved, and yet, to my knowledge, very few reports of fossil DNA surface in the literature. Also, one of the secular tests of aDNA authenticity compared the target sequence to chicken DNA, based on the belief that since dinosaurs evolved into birds, they should share a similar sequence (Morell, 1993). Woodward et al. (1994) did not match the DNA to chicken, so his discovery was supposed to have been labeled "anomalous" instead of dinosaurian. But with such an overtly evolution biased protocol, and assuming the source dinosaur was fossilized only thousands of years ago during Noah's Flood, Woodward's team may have sequenced genuine dinosaur DNA. We may never know for sure, but one vote against Woodward's DNA being dinosaurian came in a report by Hedges and Schweitzer (1995) showing that its 133 bp-long sequence of mitochondrial cytochrome b gene clustered more closely with mammals than nonmammals.

Woodward suffered open ostracism from the science community, so it is tempting to attribute the dearth of dinosaur or other Mesozoic fossil DNA reports over the last few decades to avoidance of attempts to search for it. Does the fear of ostracism or the actual absence of dinosaur DNA from fossils play the largest role in its scarcity in scientific literature? Why search for something that will only threaten one's livelihood? Perhaps it was for this kind of professional pressure, documented by Bergman (2008), that the next report of dinosaur DNA fell short of specifically calling it of dinosaur origin. Instead, the coauthors merely noted that a chemical signature matching that of DNA was found inside Cretaceous *Tyrannosaurus rex* osteocytes. We mention other biochemicals from this report in the next section (Schweitzer et al., 2013a). This *T. rex* "DNA" could have been sequenced to discern its authenticity, but was not. Perhaps the painfully contentious nature of Mesozoic DNA continues to dampen researchers' enthusiasm for definitely identifying dinosaur or similar DNAs. On the other hand, creation research has nothing to fear from such discoveries, and much to gain, since DNA should never be expected to last a million years under any reasonable earth surface environment.

DNA's half-life is short enough to clearly confront Mesozoic-dated DNAs as well as to challenge reported dates of Cenozoic aDNAs. As examples, a string of reports from Europe's supposedly oldest human remains from the Sima de los Huesos cave system in Spain cite ages that hover around 700,000 years, whereas the Allentoft data place an outer limit on DNA longevity at that cave's temperature of 10.6C of approximately 350,000 years (Thomas and Tomkins, 2014). Secular reports of aDNA from Holocene deposits actually often fit within the expected shelf life for DNA predicted by Allentoft et al. (2012). Thus, each report needs sifting to determine whether or not it presents genuinely endogenous DNA, and if it does, whether or not DNA's half-life refutes that aDNA's secular age assignment.

Mary Schweitzer's Findings

The few scientists or laypersons who have heard whispers of fossil tissues seem only to be aware of one find—a claim by Mary Schweitzer of North Carolina State University that soft, pliable tissue was found in a *Tyrannosaurus rex* femur (Schweitzer et al., 2005; see Figure 1). Unaware, for whatever reasons, of other similar discoveries, many secularists find it easier to dismiss this claim as a singular anomaly by merely arguing that a sample size of one is too small to support a far-reaching conclusion such as that these tissues are genuine. They have argued, for example, that this supposed anomaly might be explained as bacterial contamination, for example mucilaginous bacterial biofilm (Kaye et al., 2008; Anderson, 2015). In a private conversation, one Smithsonian paleontologist even suggested that the investigators probably mistook contamination from a field worker's lunch for soft, pliable connective tissue including blood vessels and erythrocyte-like elements.

Figure 1. Unarticulated hadrosaur vertebra found in northern South Dakota in 2012 from the Hell Creek Formation, the same formation that harbors Mary Schweitzer's original dinosaur tissue fossil finds. Photo by Paul Koepp.

Sheer time delay caused by the ensuing debate over whether researchers found original soft tissue or some kind of mistake probably blunted the impact that even the splashy *T. rex* report could have had if secular scientists had readily accepted its conclusions upon publication. This debate has also offered a seemingly easy exit for those less willing to admit to the reality of Mesozoic original tissue fossils—namely, that by declaring without explanation that the results are inconclusive—we must wait on further investigations to settle the matter.

One is reminded at this point of origin of life research, wherein evolutionary biologists admit that abiogenesis has its difficulties, but show faith that biochemists are working on the problem and one day will solve it. In reality, however, those biochemists have merely encountered a myriad of chemical hurdles to abiogenesis. Left alone, complicated biochemicals essential to life, like proteins and nucleic acids, spontaneously degrade into smaller, simpler components, but abiogenesis requires nature to do just the opposite. In other words, science has proven beyond a reasonable doubt that originating life without a Creator is impossible. But rather than admit this truth and accept its theological consequences, run-of-the-mill secularists sometimes skirt the whole discussion and at worst purpose-fully maintain ignorance of it so they can deceive themselves into thinking that origin of life research still has hope. The same laws of chemical degradation that ensure biomolecules fall apart instead of self-assemble and thus refute abiogenesis also explain why original tissue fossils look like recent deposits. After a million years they should all have disintegrated completely. So, for the same reasons that all researchers should embrace the science that disproves abiogenesis, they should likewise embrace the science that disapproves millions of years for fossils, including original tissues.

Calling on more research is no longer a valid excuse. The necessary investigations have now run their courses, proving beyond all reasonable doubt that the *Tyrannosaurus* tissues were genuine and not contaminations. First, Schweitzer and colleagues sequenced collagen protein from the dinosaur fossil, found in Montana's Hell Creek Formation, and designated Cretaceous (Organ et al., 2008). Collagen protein is an essential and integral component of bone, epithelial, and other vertebrate tissues, but microbes are not known to manufacture it (see Anderson, 2015).

Second, Schweitzer et al. (2013a) published a more detailed analysis of her *Tyrannosaurus*' biochemicals, using immunofluorescence as a primary detection tool. Accordingly, antibodies of those specific biochemicals are applied to a small slice of acid-demineralized bone tissue. If the target biochemical is present, the antibody adheres to it. Unbound antibodies wash off. Fluorescent markers are then applied to the samples, designed to attach to the other end of the antibody molecules. The resulting image shows fluorescent patches only where the target biochemical occurs in the fossil. Schweitzer and colleagues imaged proteins specific to vertebrates, including PHEX and histone H4 (Schweitzer et al., 2013a). She also coauthored a paper describing many of the same vertebrate-specific proteins in a Hell Creek Formation hadrosaur femur, being careful to collaborate with an outside laboratory that sequenced some of the hadrosaur collagen, including elastin and laminin (Schweitzer et al., 2009).

Third, although her very thorough work should put the nail in the coffin of bacterial contamination objections, other authors have added new research that bolsters the conclusion of genuine original dinosaur tissue fossils in general. Anderson and Armitage published their remarkable discovery of a large, soft and pliable whitish sheet of fibrillar tissue integral to a bony *Triceratops* horn core, also collected from the Hell Creek Formation (Armitage and Anderson, 2013; Armitage, 2015). Whether or not most evolutionists choose to assent to the fossil tissue data championed by Schweitzer, the data unambiguously demonstrate the parsimony of her conclusion that the fossil biomaterials are endogenous, as further illustrated in the next section.

Fossil Tissue in Mesozoic Strata

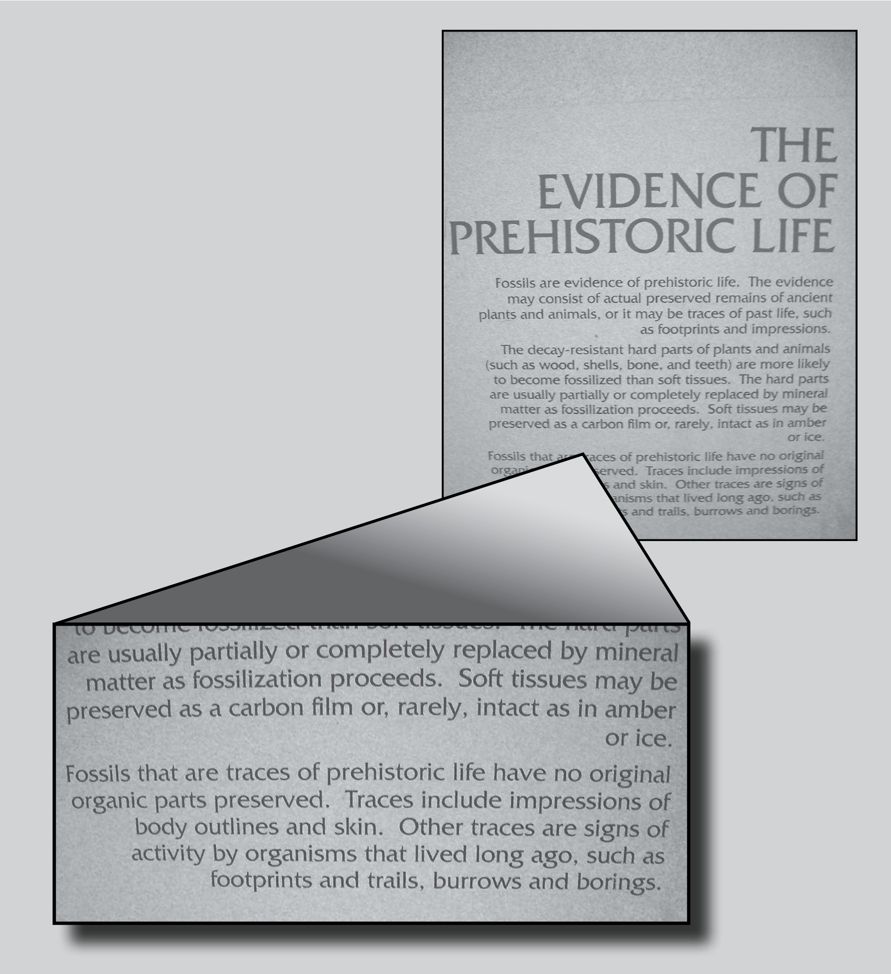
Perhaps the best-preserved fossils with probably the highest number of original tissue reports describe Cenozoic fossils. For example, just one Siberian mammoth bone yielded 126 unique, partly intact protein types, detected by tandem mass spectroscopy (Capellini et al., 2012). However, we restrict the current discussion to original tissue fossils from much lower geologic strata because they intensify the strain between secular age assignments and age expectations that follow from biblical creation. Mesozoic strata supposedly straddle an evolutionary time range of 252 to 66 million years. Many and perhaps the most intriguing original tissue fossil discoveries hail from the Mesozoic Erathem.

Many who begin investigating this issue are surprised to find that the scientific literature has for decades been sprinkled with original tissue fossil discoveries. Long before Schweitzer's spectacularly bloody *T. rex*, investigators had been describing dinosaur proteins and proteinaceous bone structures. One early detection tool was electron microscopy. Top British journal *Nature* published electron micrographs of dinosaur tissue in 1966 (Pawlicki et al., 1966). A group of paleontologists applied the technique in 2008 to exceptionally well-preserved *Psittacosaurus* remains from China, publishing images of dinosaur collagen fiber bundles (Lingham-Soliar, 2008). South African researcher Theagarten Lingham-Soliar published stunning skin color images from a separate *Psittacosaurus*, also from China, finding evidence of original, unaltered pigments including carotenoids and melanins (Lingham-Soliar and Plodowski, 2010).

Lindgren et al. (2010) described scale skin and hemoglobin decay products—still colored red as were some of Schweitzer's *T. rex* and hadrosaurine samples—in a Kansas mosasaur. Researchers working in southern China reported endogenous protein from a *Lufengosaurus* bone taken from the tiny Jurassic sauropod's embryo (Reisz et al., 2013).

Polish researcher Roman Pawlicki's scientific output detailing original dinosaur tissues spanned more than three decades, and included exquisite electron micrographs of bone tissue from a Gobi Desert *Tarbosaurus bataar* in 1998, for example. The report noted, "the descriptions presented confirm that the morphology of the vascular canals in dinosaur bones and the bones of modern reptiles is the same" (Pawlicki and Wowogrodzka-Zagorska, 1998, p. 76). It appears this is the same Tarbosaurus that he had imaged in 1978 (Pawlicki, 1978). A string of papers from Pawlicki and various coauthors, dating back to at least 1966, also imaged original cells, chemically verified original collagen (Pawlicki et al., 1966) and even reported an immunoassay detecting DNA in dinosaur osteocytes (Pawlicki, 1995). Armitage verified fresh-looking tissues by generating high quality SEM images of dinosaur bone in 2001 (Armitage, 2001). This important research verified for and brought under the umbrella of the creation science community what secular researchers had been publishing. Two years later a separate team sequenced some non-collagen protein fragments from an *Iguanodon* bone housed at the Natural History Museum of London (Embery et al., 2003). This list does not exhaust research on the topic, but serves to illustrate the repeatability of finding endogenous, minimally altered biomaterials.

Thus, long before Schweitzer's work, researchers had described amino acids from dinosaur bone, including a New Mexico *Seismosaurus* (Gurley et al., 1991) and even in fossil shells (Akiyama and Wyckoff, 1970). Note the significant contrast between the statement from *Biogeochemistry of Amino Acids* (published in 1980 and partly sponsored by the Carnegie Institution of Washington) that declares "work with dinosaur remains demonstrated that enough protein for analysis could often be recovered from bones and teeth as old as the Jurassic" (Wyckoff, 1980, p. 19), and the statement which currently appears on a sign at the entrance to the paleontology exhibit at the Carnegie museum in Pittsburgh that comments "fossils that are traces of prehistoric life have no original organic parts preserved." These two statements could not stand in more abject opposition (see Figure 2).



THE
EVIDENCE OF
PREHISTORIC LIFE

Fossils are evidence of prehistoric life. The evidence may consist of actual preserved remains of ancient plants and animals, or it may be traces of past life, such as footprints and impressions.

The decay-resistant hard parts of plants and animals (such as wood, shells, bone, and teeth) are more likely to become fossilized than soft tissues. The hard parts are usually partially or completely replaced by mineral matter as fossilization proceeds. Soft tissues may be preserved as a carbon film or, rarely, intact as in amber or ice.

Fossils that ar races of prehistoric life have no original organi served. Traces include impressions of s and skin. Other traces are signs of anisms that lived long ago, such as s and trails, burrows and borings.

are usually partially or completely replaced by mineral matter as fossilization proceeds. Soft tissues may be preserved as a carbon film or, rarely, intact as in amber or ice.

Fossils that are traces of prehistoric life have no original organic parts preserved. Traces include impressions of body outlines and skin. Other traces are signs of activity by organisms that lived long ago, such as footprints and trails, burrows and borings.

Figure 2 (a and b). Incorrect information on a sign in the Carnegie Museum of Natural History in Pittsburgh. Photo by Brian Thomas.

Fossil Tissue in Paleozoic Strata

Paleozoic original tissues seem to be less well represented in the literature than Mesozoic or Cenozoic finds, but there are some examples. Their lower numbers in lower strata could be an artifact of investigator bias. After all, secularists have a difficult enough time imagining that original tissues somehow lasted tens of millions of years, so they generally would not imagine that original tissues could preserve for hundreds of millions of years.

What expectations would a Flood geology perspective bring to this issue? Flood geologists maintain that almost all Mesozoic and Paleozoic strata derive from Noah's Flood. By considering this factor in isolation, one might expect fossils from any Flood stratum to have been deposited at virtually the same time only 4500 or so years ago, and therefore should contain virtually equal numbers of original tissue fossils. Indeed, Baumgardner et al. (2003) found striking concordance between carbon ages for Mesozoic and Paleozoic coals. However, many Paleozoic strata in particular appear to have experienced greater temperatures. Even a brief high temperature episode could greatly accelerate biochemical decay, and if temperatures reach a certain height, facilitate biomolecular reorganization or alteration. As an example, Burgess Shales include exceptional preservation of Cambrian marine paleofauna, but their organic content has largely been transformed into thin, carbonaceous residues. Many organics from this loca-

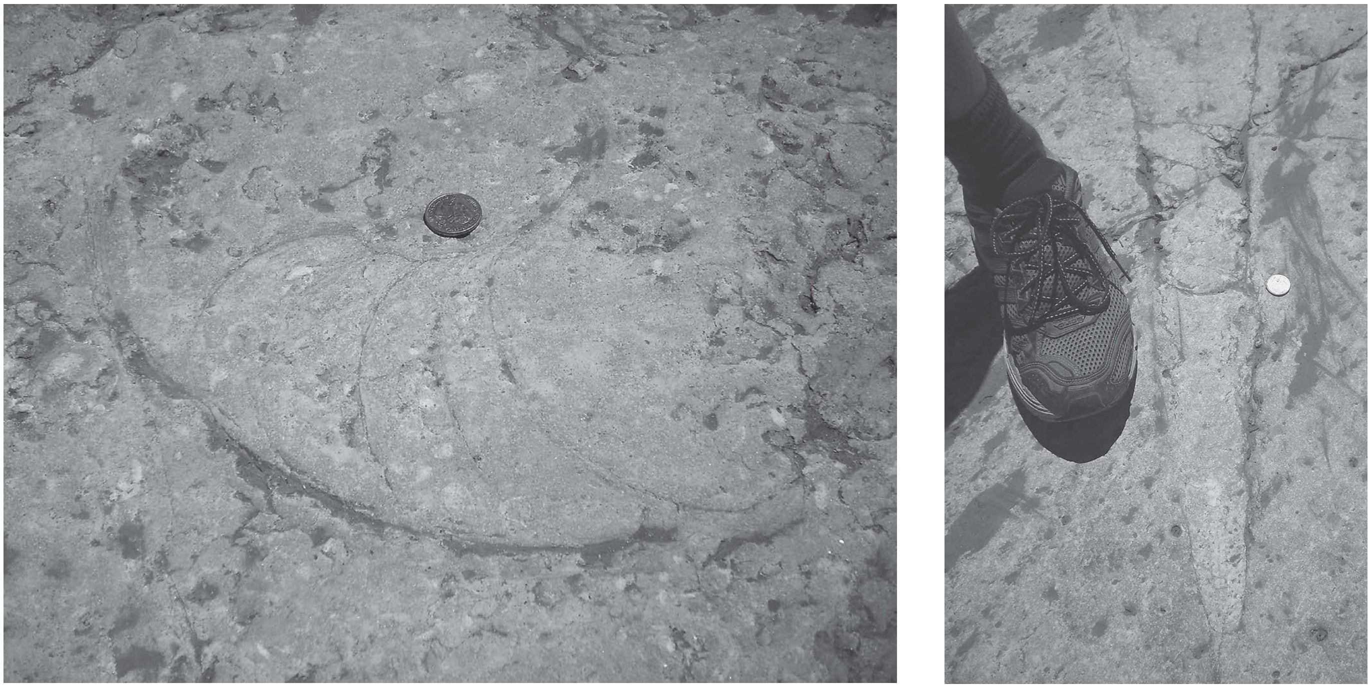


Figure 3 (a and b). Ancient hydrothermal fluids completely dissolved, transformed, and/or replaced all original tissues from nautiloids found in the Redwall limestone, here exposed at Grand Canyon. Photos by Brian Thomas.

tion and others have been kerogenized, a taphonomic process whereby excess heat cross-links biochemicals into a decay-resistant material, kerogen.

One spectacular exception came from a German and Russian team's recent investigation of a *Vauxia gracilenta* sea sponge fossil from British Columbia's famous Burgess Shale deposits (Ehrlich, et al., 2013). They searched for, but failed to find, endogenous DNA. Uniformitarians expect its absence on the basis of their age assignment, and catastrophists expect its absence on the basis of the elevated temperatures that its rocks indicate once occurred there. But the team identified intact chitin using a half dozen different techniques including fluorescence microscopy, fourier transform infrared microscopy, high-performance capillary electrophoresis, high-pressure liquid chromatography, mass spectroscopy, and others. Chitin is a biochemical found in squid beaks and pens, arthropod exoskeletons, and certain fungi. In sea sponge support structures, chitin incorporates tiny glass-like spicules. Experimentally determining a chitin decay rate could add a new and very valuable biochemical clock against which to judge conventional age assignments of chitinous fossils like this. The study authors noted the mixed results from attempts to estimate chitin's longevity before plainly admitting that the mechanism for Cambrian chitin preservation is simply unknown.

Geologic observations of heated Paleozoic strata, for example radiohalos caused by thermal fluid transport of uranium decay daughter products (Snelling, 2005) and metamorphosed rock on the margins of hydrothermal pipes that penetrate sedimentary layers, clearly indicate ancient heating. Paleozoic nautiloid fossils from the base of the Redwall Limestone described by Austin preserve no original tissues (Austin, 1994, p. 27; see Figure 3). The nautiloids' soft body parts do not occur *in situ*, and even their shell material has been replaced by hydrothermal fluid-derived mineralization.

Flood geologists generally relate these phenomena, as well as voluminous paleovolcanism like the Siberian and Deccan traps, to Genesis 7:11. Because many of the earliest Flood deposits, which generally correspond to the Paleozoic Erathem, were exposed to more heat for a longer time than later Flood deposits, Flood geologists would generally not expect them to yield as many unaltered original tissue fossils as from upper layers that may not have been exposed to as much heat for as many days or months during the Flood. However, not all places on Earth were affected in the same ways. Differential heating leaves open the possibility that rare Paleozoic zones remained cool enough to permit original tissue fossil preservation through the torturous Flood year until today, and indeed several remarkable cases have been reported, including the Burgess *Vauxia* sponge already mentioned. Of course, other factors also must exist for any of these biomaterials to persist longer than hundreds of years or so, most notably the absence or diminishment of biodegrading microbes.

Ordovician graptolite periderm exhibited collagen-like structures, imaged by wide-angle X-ray diffraction in 1972 (Towe and Urbankek, 1972). The researchers found a few amino acids, but not 4-hydroxyproline or 5-hydroxylysine, which characterize collagen. X-ray diffraction did reveal helical fibers consistent with collagen's triple helix structure. Their results were thus not definitive for original collagen, since perhaps minerals could somehow have replaced the collagen and preserved only its molecular shape, but were consistent enough with the hypothesis of original collagen to warrant further investigation. Spectacularly preserved Paleozoic scorpion and false scorpion fossils retained their original exoskeletons, as chemical analyses revealed chitin and chitin-associated protein (Cody et al., 2011).

The possibility of endogenous biochemistry in early Cambrian fossils from China's Chengjiang biota, perhaps the world's best-preserved Cambrian Lagerstatten, has not been settled in the literature. Nevertheless, several features of some of these supposedly 520 million-year-old fossils strongly suggest they retain original tissues. One nontrilobite arthropod *Cindarella eucalla* showed obvious dark coloration, which in more recently deposited fossils (whose biochemistry *has* been tested consistently) indicates endogenous pigmentation such as melanins. It preserved 1,000 ommatidia on the half of its eye that was exposed. Compound eyes are composed of cone-shaped units called ommatidia, each equipped with a cornea, light-sensitive cells, and an optic nerve. "A thin film overlying the eye indicates the presence of an eye exocuticle, the film shows wrinkles and tears indicating the soft tissue shrank during initial burial" (Zhao et al., 2013, p. 2751).

Research into the mode of taphonomy preserving the Chengjiang biota has not yet adequately resolved the modes of preservation in my estimation, and controversy persists in the literature. Some authors seem to slap a glib and hasty explanation into their reports, for example claiming preservation by silicification, phosphatization, carbonization, pyritization, phyllosilicate metamorphism, or apatite permineralization—all processes known to contribute to fossilization in some specimens—but do not report positive tests to support such claims. As an aside, some of these modes of preservation counterintuitively involve bacterial degradative action across a soft tissue organ or other surface. The resulting carbon dioxide waste acidifies the microenvironmnent to which a preserving layer of mineral adheres. Other reports do not even attempt to answer taphonomic preservation questions, instead burrowing into a certain fossil's anatomical details, or possible phylogenies.

Burgess Shale expert Derek Briggs coauthored a review of Chengjian biota, saying,

> Elemental mapping of fossils from the Maotianshan Shale revealed that two modes of preservation are important in that deposit. In most cases the major morphological features of Chengjiang fossils are preserved as carbonaceous compressions; however, features of many of these fossils are preserved in pyrite. (Gaines et al., 2008, p. 755)

Pyrite is not original tissue, but "carbonaceous compressions" might be. They could signify some kind of kerogenization via heating, as discussed above. Alternatively, it might signify decayed remnants of original biochemistry like that verified in many other Mesozoic and Cenozoic settings. Future research may reveal whether or not these Paleozoic features consist entirely or partly of postmortem mineralization or of original biochemistry, but the high quality of preservation is consistent with the hypothesis that Chengjiang biota may preserve some original biomaterial.

Fossil Tissue in Ediacaran Source

The record for oldest evolutionary age assignment for original tissue fossils, to this author's knowledge, so far belongs to still-flexible, proteinaceous marine tube worm tubes taken from Siberian drill core samples of Ediacaran strata (Moczydlowska et al., 2014). The study authors were explicit in describing the worm casings as not mineralized, and original to the worms, as comparisons revealed virtual identity with the chitin-structural protein composite of worm casing seen in its "living fossil" counterpart, sea floor worms in the family Siboglinidae. Any objective viewer should meet the age assignment of 520 million supposed years for these still-soft worm casings with skepticism, if not incredulity.

Reactions to Discoveries

Why do so few scientists seem acquainted with this rich vintage of scientific reports of original tissue fossils? When Schweitzer's *T. rex* tissue publications began circulating, why did most secular scientists seem to act as if this was the first of its kind? The answers to these questions may be perpetually relegated to speculation, but a few possibilities present themselves.

First, Schwietzer's *Science* report pioneered visceral color images. Prior reports showed black-and-white electron micrographs, black-and-white hand-drawn sketches, charts of amino acid content, two-tone immunoassay images,

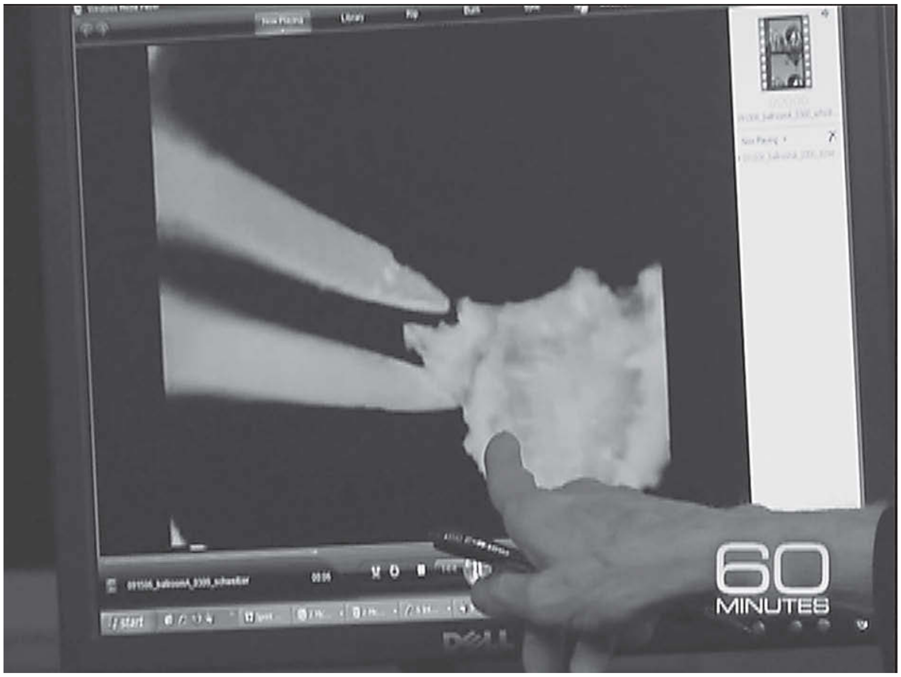


Figure 4. CBS News Anchor Leslie Stahl points to Mary Schweitzer's computer screen, showing video of rebounding elastic tissue taken from a *Tyrannosaurus rex* fossil. "B-Rex" episode on *60 Minutes*, aired November 15, 2009, posted on cbsnews.com/videos/b-rex/

plus many words. Such means of communication simply cannot convey what color photographs can, and although the image-rich Schweitzer et al. (2005) *Science* paper did not include video, the *T. rex* tissues were video recorded and this kind of footage adds a whole new dimension of communication. This is clearly exemplified by watching CBS's *60 Minutes* program titled "B-Rex" (see Figure 4).

Scientists are people too, so great images can make great impressions on them just as on others. The first blood-red *T. rex* pictures to be published undoubtedly made their way to a wider viewing audience than previous reports of original tissue fossils. The image said it all. No words were necessary. Even nonscientists became intrigued, and experts from outside the discipline of paleontology finally intersected with the story of original biomaterial fossils.

Paleontologists are not necessarily trained in biochemistry deeply enough to intuitively realize that proteins and DNA cannot last a million years. So as they were discovering and describing these biomolecules in fossils in earlier decades, mostly for the benefit of other paleontologists, the chances that biochemists came across and evaluated the results may have been much smaller. Some who happened to run across such reports reasoned, without hiccup, that since these biochemicals were found in fossils, and since the fossils were millions of years old, biochemicals can obviously last millions of years. Perhaps protein decay rates were not obvious to those who used this logic. It wasn't until Schweitzer's *T. rex* images that biochemists seem to have become aware of what paleontologists had been uncovering, and at that point they began expressing dissent. Fierce debate ensued.

In particular, *Science* magazine published a letter by Buckley et al. (2008) that included Michael Collins as a coauthor offering a rebuttal to Asara et al.'s 2007 *T. rex Science* paper. These researchers argued that whatever Schweitzer's lab found in that *T. rex* femur, it was most likely not original bone collagen, since their lab results clearly limit collagen's maximum longevity to fewer than one million years assuming reasonable earth surface temperatures. Michael Collins runs one of the world's few archaeobiology labs, specializing in biochemical decay rates. As discussed above, Collins and colleague's repeated tests have supplied maximum shelf lives for bone collagen and bone DNA that are incongruent with the naive conclusion that biomaterials must somehow simply persist across deep time. Currently, the evolutionist community (including theistic) enjoys no consensus on answering the questions surrounding endogenous fossil biochemistry. A gamut of explanations and excuses continues to troll the intellectual seascape. They include stories that cannot accommodate the data, such as sheer belief that (1) biochemical molecules last longer than studies show, (2) that there is no such thing as soft-tissue-containing fossils, or (3) that all specimens are merely bacterial contaminants.

In sum, it seems plausible that color photographs showing obvious fresh-looking original tissue fossils drew enough attention that for the first time biochemistry-minded researchers became aware of these finds, and this may account for their broader impact. If a broader spectrum of investigators had been scouring paleontological reports, these biochemists could probably have expressed their dissent decades ago. Despite newfound attention, both casual and serious researchers have not all answered some of the fundamental questions that these original biomaterials generate.

Proposed Mechanisms of Preservation

At least three reports have described attempts to explain biochemical longevity within the framework of deep time, and none of them withstand close scrutiny.

Smectite Adhesion

In the first report under consideration, researchers used six different techniques to verify original keratin protein in a lizard skin fossil from the Green River Formation (Edwards et al., 2011). Knowing that keratin could not last for over 50 million years without some very significant (essentially miraculous) external help, the team speculated that smectite minerals in the clay-rich matrix adhered to and stabilized proteins to preserve the whole skin structure. Whereas smectites do have amazing properties including the ability to act like molecular tubes that entrap smaller molecules, this explanation suffers from several shortcomings.

First, the authors did not discuss how smectites could be transported onto the skin, but this must have occurred via water flow. The presence of water accelerates biochemical decay directly by oxidation and indirectly by facilitating other degradative chemistry and by enabling microbial growth. The authors ignore these considerations. Second, they mapped keratin's sulfurous composition on lizard scales, so their model requires an exceedingly unlikely scenario of the fossil remaining dry for 40 million years after it was wet enough to bring in smectites. Sulfur is water soluble and should have been removed long ago by ground water percolation under a scenario of evolutionary time. Essentially, their model is very unlikely because it invokes a set of implausible conditions.

Apatite Sequestration

In the literature, Mary Schweitzer has not followed the original fossil tissue evidence to the logical conclusion that dinosaur and other fossils were deposited thousands, not millions of years ago. One of her workarounds, coauthored by Mike Buckley who had rejected her initial conclusions in *Science* along with Collins, argued that bone collagen could last millions of years on the basis of bone collagen ultrastructure and its interaction with bioapatite (San Antonio et al., 2011). Accordingly, because collagen must expand in order to degrade, and because bioapatite is so tightly packed around each collagen molecule that it prevents collagen from expanding, the collagen can supposedly last as long as the apatite persists.

However, in making their case, these authors fell prey to a common circular argument. They first asserted that their observations of collagen ultrastructure support their belief that collagen could last millions of years. Then, they argued that because the dinosaur fossils from which they obtained collagen were deposited millions of years ago, the experimentally determined decay rates—which they conveniently downgraded to decay "models"—must be in error. In the end, they simply dismissed the collagen decay experiments and backfilled their "knowledge" that collagen must have persisted over deep time with a convenient story. In reality, the decay experiments already took into account collagen ultrastructure and its interaction with bioapatite, and thus already represent a best-case preservation scenario. If apatite sequestration really could preserve collagen for 180 million years—the oldest secular age assignment for it of which I am aware—then apatite would have preserved collagen in the decay experiments virtually indefinitely, but it did not.

Iron Adhesion

Apparently satisfied that she had answered the question of how collagen could have lasted for millions of years, Schweitzer next tackled the question of preserving whole tissues such as blood vessels. Her coauthored paper reported that when placed in homogenized and concentrated blood, bird blood vessels in bone did not appreciably decay even after being held at room temperature for two years (Schweitzer et al., 2013b). On this basis, the authors speculated that blood-derived iron adhered to the inner and outer surfaces of dinosaur blood vessels to preserve them for millions of years. While certainly informative, the bird tissue results fall short of explaining the original tissue fossil data for three reasons.

First, without an actual decay rate of vascular tissue in blood concentrate, we cannot reliably extrapolate an age expectation. Two years is too short a time, even at room temperature, from which to draw these authors' conclusion. Second, not only did the dinosaur soft tissue in bone include collagen protein and blood vessels, but also whole osteocytes with several vertebrate-specific proteins still intact inside them. Osteocytes do not behave like collagen, so apatite sequestration should not be invoked to explain their preservation. But the tiny points of access to osteocytes in bone like canaliculi have too small a diameter to permit the imaginary blood concentrate access. They are much smaller than capillary tubes. So, even if blood-derived iron can preserve, it does no good if it cannot reach the cells in question. Last, many original biomaterial fossils occur in virtually bloodless settings. For example, no evidence for ancient blood baths presents itself in most of the fossils described above, such as dinosaur skin or Sabellidae worm casings. By far the most straightforward explanation of original tissue fossil preservation involves reassigning their ages from millions down to thousands of years (Demassa and Boudreaux, 2015).

Future Research Directions

As above discussions hint, opportunities for researching original biomaterial fossils abound. For example, a huge quan-

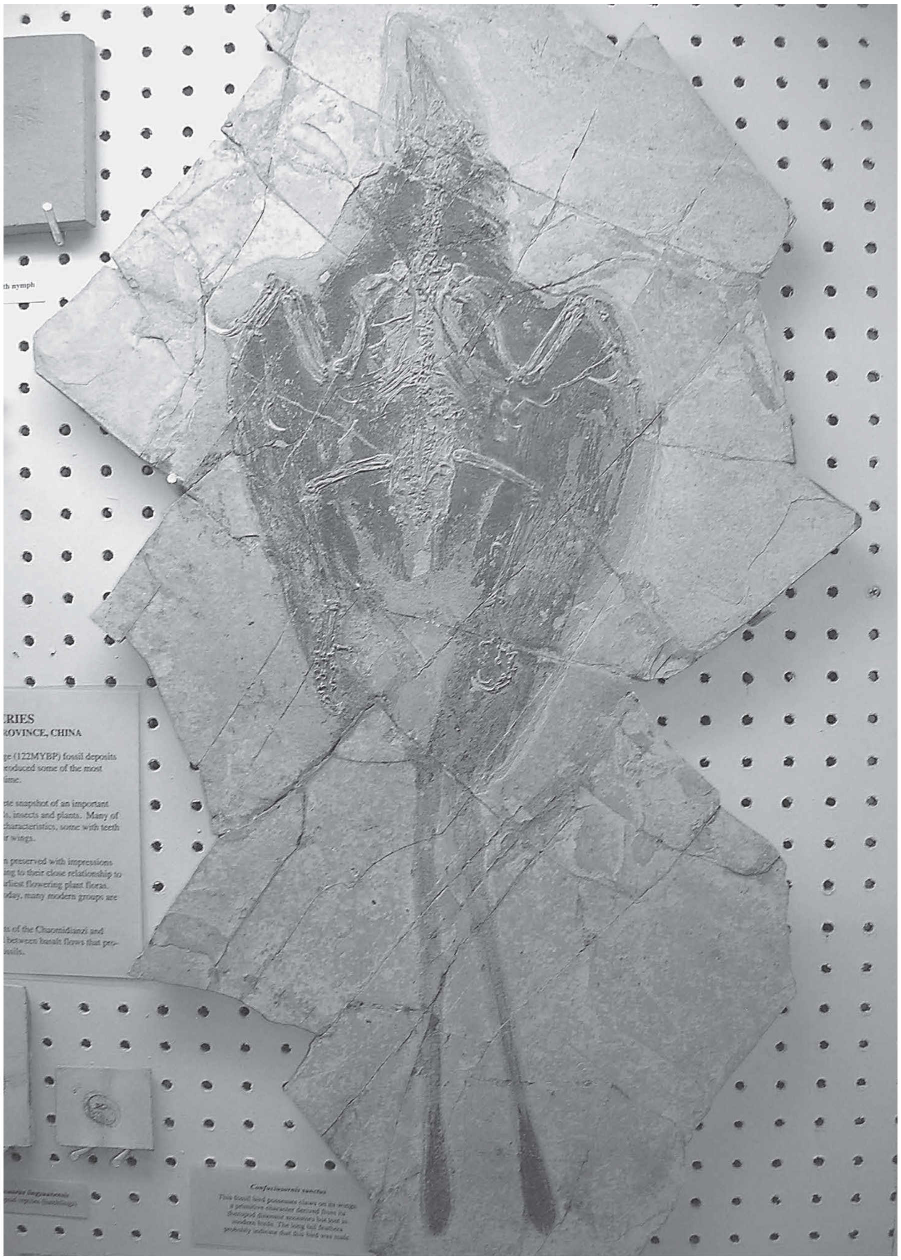


Figure 5. A darkened halo, perhaps often consisting of original pigmentation, surrounds many fossils, like the plumage surrounding this *Confuciusornis sanctus* extinct bird fossil displayed at the Museum at Black Hills Institute, Hill City, South Dakota. Photo by Brian Thomas.

tity of fossils seem to preserve original pigmentation, including famous ones like "Ida" from Messel shale, several mosasaurs, and a supposedly feathered dinosaur *Sinosauropteryx*, as well as more mundane finds like Cenozoic penguin feathers and Mesozoic bird feathers from Brazil. Many fossils retain a dark-colored halo of pigmentation that paleontologists increasingly recognize—in part thanks to the increased attention that Schweitzer has brought to biogeochemistry—originates from original biological pigmentation (see Figure 5).

Pigments like melanins are very resistant biological materials, and might be expected to outlast DNA and proteins, but could they really last for millions of years? Taphonomic scenarios need to be proposed and tested, and decay rates for melanins should be experimentally established if we are to gain a firm understanding of these relatively common fossil biochemicals.

Similarly, chitin is another long-lasting biopolymer, but just how long is not yet known with experimental certainty. An empirically derived decay rate for chitin could open expansive new areas of research into the potential longevity of fossilized chitin, which has been reported—especially that of fungal chitin—as occurring throughout the fossil record.

Additional work needs to occur to more precisely understand DNA in fossils. For example, by applying a corrective algorithm to carbon dates, one could adjust downward to a more realistic rate the published half-life of the control region of ancient mitochondrial DNA (Thomas and Tomkins, 2014). Other aDNAs, most notably mammalian chromosomal DNA in light of the numerous ancient genomes for genera like *Homo*, *Ursa*, and *Equus* as examples, should also be subjected to decay analyses. Also, as noted above, creation researchers should increase attempts to detect aDNA, especially from amber, bone, and tooth fossils, since its discovery would be consistent with the creation model prediction that some aDNAs might still be detectable after only several thousand years have elapsed.

Finally, future research into original biomaterial fossils would benefit from more precise terminology, and investigators cannot successfully engage in this research without carefully sifting through literature. The *Nature* paper describing "exceptional preservation" in a Cambrian nontrilobite arthropod (Reisz et al.,

2013) exemplifies this need for careful sifting. A colleague forwarded the paper to me under the impression that it presented original tissue fossil data, but the report did not verify any biomaterial. It cited "non-biomineralized compound eyes," not "non-mineralized," merely indicating that the creature's compound eye ommatidia were simply somehow preserved (Zhao et al., 2013). One last example also illustrates the potential for confusion and thus the need to vet literature that uses phrases like "remarkable preservation" and even "soft tissue" to mean both original or mineralized fossils typifies scores of similar reports. Its title, "Soft-tissue preservation in the Lower Cambrian linguloid brachiopod from South China" might give the impression to those who only read headlines of endogenous biomaterials, but the report permits "early phosphatic mineralization and subsequent replacement by the clay minerals may also have played a taphonomic role in a way analogous to the Burgess Shale" as a means of preservation (Zhang et al., 2004, p. 261).

Conclusions

In his brief review of original tissue fossils, creation researcher Mike Oard wrote,

> It truly is astonishing that bacteria, DNA, red blood cells, bone proteins, etc. could really survive the vicissitudes of tectonics, heating events, water seeping through the rocks, and other geological processes for millions of years and remain 'alive.' Before this barrage of discoveries, scientists considered that such survival was impossible past several thousand or tens of thousands of years. The evidence sits much more comfortably within the young-Earth Creation/Flood model. (Oard, 2001)

And this evidence continues to accumulate into the body of technical literature. Plenty of original biomaterial finds have firmly established proteins endogenous to Cenozoic and Mesozoic fossils, plus a few finds in lower layers, and each carries with it a strong implication of a recent creation and Flood.

References

Akiyama, M., and R.W.G. Wyckoff. 1970. The total amino acid content of fossil pecten shells. *Proceedings of the National Academy of Sciences* 67:1097–1100.

Allentoft, M.E., M. Collins, D. Harker, J. Haile, C.L. Oskam, M.L. Hale, P.F. Campos, J. A. Samaniego, M.T.P. Gilbert, E. Willerslev, G. Zhang, R.P. Scofield, R.N. Holdaway, and M. Bunce. 2012. The half-life of DNA in bone: measuring decay kinetics in 158 dated fossils. *Proceedings of the Royal Society B: Biological Sciences* 279:4724–33.

Anderson, K. 2015. Dinosaur tissue or bacterial biofilms? *Creation Research Society Quarterly* 51:259–267.

Armitage, M. 2001. Scanning electron microscope study of mummified collagen fibers in fossil Tyrannosaurus rex bone. *Creation Research Society Quarterly* 38:61–66.

Armitage, M. 2015. Soft bone material from a brow horn of a *Triceratops horridus* from Hell Creek Formation, MT. *Creation Research Society Quarterly* 51:248–258.

Armitage, M.H., and K.L. Anderson. 2013. Soft sheets of fibrillar bone from a fossil of the supraorbital horn of the dinosaur Triceratops horridus. *Acta Histochemica* 115(6): 603–608.

Asara, J. M., M.H. Schweitzer, L.M. Freimark, M., Phillips, L.C. Cantley. 2007. Protein sequences from mastodon and *Tyrannosaurus rex* revealed by mass spectrometry. *Science* 316: 280–285.

Austin, J. n.d. The search for DNA in amber. The Natural History Museum of London fact sheet. http://www.nhm.ac.uk/nature-online/earth/fossils/dna-amber/ (accessed November 4, 2014).

Austin, S.A. 1994. *Grand Canyon: Monument to Catastrophe*. Institute for Creation Research, Santee, CA.

Baumgardner, J.R., A.A. Snelling, D.R. Humphreys, and S.A. Austin. 2003. Measurable 14C in fossilized organic materials: confirming the young earth creation-flood model. In Ivey, R.L. (editor), *Proceedings of the Fifth International Conference on Creationism*, pp. 127–142. Creation Science Fellowship, Pittsburgh, PA.

Bergman, J. 2008. *Slaughter of the Dissidents*. Leafcutter Press, Southworth, WA.

Buckley, M., and M.J. Collins. 2011. Collagen survival and its use for species identification in Holocene-Lower Pleistocene bone fragments from British archaeological and paleontological sites. *Antiqua* 1:e1.

Buckley, M., A. Walker, S.Y.W. Ho, Y. Yang, C. Smith, P. Ashton, J.H. Oates, E. Cappellini, H. Koon, K. Penkman, B. Elsworth, D. Ashford, C. Solazzo, P. Andrews, J. Strahler, B. Shapiro, P. Ostrom, H. Gandhi, W. Miller, B. Raney, M.I. Zylber, M.T.P. Gilbert, R.V. Prigodich, M. Ryan, K.F Rijsdijk, A. Janoo, and M.J. Collins. 2008. Comment on "Protein sequences from Mastodon and *Tyrannosaurus rex* revealed by mass spectrometry." *Science* 319: 33c.

Cano, R.J., H.N. Poinar, N.J. Pieniaze, A. Acra, and G.O. Poinar Jr. 1993. Amplification and sequencing of DNA from a 120–135-million-year-old weevil. *Nature* 363:536–538.

Cappellini, E., L.J. Jensen, D. Szklarczyk, A Ginolhac, R.A.R. da Fonseca, T.W. Stafford Jr., S.R. Holen, M.J.Collins, L.Orlando, E. Willerslev, M.T.P. Gilbert, and J.V. Olsen. 2012. Proteomic analysis of a Pleistocene mammoth femur reveals more than one hundred ancient bone proteins. *Journal of Proteome Research* 11:917–926.

Cody, G.D., N.S. Gupta, D.E.G. Briggs, A.L.D. Kilcoyne, R.E. Summons, F. Kenig, R.E. Plotnick, and A. C. Scott. 2011. Molecular signature of chitin-protein complex in Paleozoic arthropods. *Geology* 39(3): 255–258.

Demassa, J.M., and E. Boudreaux. 2015. Dinosaur peptides: preservation and degradation. *Creation Research Society*

Quarterly 51:268–285.

Edwards, N.P., H.E. Barden, B.E. van Dongen, P.L. Manning, P.O. Larson, U. Bergmann, W.I. Sellers, and R.A. Wogelius. 2011. Infrared mapping resolves soft tissue preservation in 50 million year-old reptile skin. *Proceedings of the Royal Society B* 278:3209–3218.

Embery, G., A.C. Milner, R.J. Waddington, R.C. Hall, M.S. Langley, and A.M. Milan. 2003. Identification of Proteinaceous material in the bone of the dinosaur Iguanodon. *Connective Tissue Research* 44(suppl. 1): 41–46.

Ehrlich, H., J.K. Rigby, J.P. Botting, M.V. Tsurkan, C. Werner, P. Schwille, Z. Petrášek, A. Pisera, P. Simon, V.N. Sivkov, D.V. Vyalikh, S.L. Molodtsov, D. Kurek, M. Kammer, S. Hunoldt, R. Born, D. Stawski, A. Steinhof, V.V. Bazhenov, and T. Geisler. 2013. Discovery of 505-million-year old chitin in the basal demosponge *Vauxia gracilenta*. *Scientific Reports* 3:3497.

Gaines, R.R., D.E.G. Briggs, and Z. Yuanlong. 2008. Cambrian burgess shale-type deposits share a common mode of fossilization. *Geology* 36:755–758.

Gurley, L.R., J.G. Valdez, W.D. Spall, B.F. Smith, and D.D. Gilette. 1991. Proteins in the fossil bone of the dinosaur, Seismosaurus. *Journal of Protein Chemistry* 10:75–90.

Hedges, S.B., and M.H. Schweitzer. 1995. Detecting dinosaur DNA. *Science* 268:1191.

Kaye, T.G., Gaugler, G., and Sawlowicz, Z. 2008. Dinosaurian soft tissues interpreted as bacterial biofilms. *PLoS ONE* 3(7): e2808.

Lindgren J., W.W. Caldwell, T. Konishi, and L.M. Chiappe. 2010. Convergent evolution in aquatic tetrapods: insights from an exceptional fossil Mosasaur. *PloS ONE* 5 (8): e11998.

Lingham-Soliar, T. 2008. A unique cross section through the skin of the dinosaur Psittacosaurus from China showing a complex fibre architecture. *Proceedings of the Royal Society B: Biological Sciences* 275:775–780.

Lingham-Soliar, T., and G. Plodowski. 2010. The integument of Psittacosaurus from Liaoning Province, China: taphonomy, epidermal patterns and color of a ceratopsian dinosaur. *Naturwissenschaften* 97:479–486.

Moczydlowska, M., F. Westall, and F. Foucher. 2014. Microstructure and biogeochemistry of the organically preserved Ediacaran Metazoan Sabellidites. *Journal of Paleontology* 88:224–239.

Morell, V. 1993. Dino DNA: The hunt and the hype. *Science* 261:160.

Oard, M. 2001. Aren't 250 million year old live bacteria a bit much? Creation.com. http://creation.com/arent-250-million-year-old-live-bacteria-a-bit-much (accessed November 4, 2014).

Organ, C.L., M.H. Schweitzer, W. Zheng, L.M. Freimark, L.C. Cantley, and J.M. Asara. 2008. Molecular phylogenetics of mastodon and *Tyrannosaurus rex*. *Science* 320:499.

Pawlicki, R., A. Korbel, and H. Kubiak. 1966. Cells, collagen fibrils and vessels in dinosaur bone. *Nature* 211:655–657.

Pawlicki, R., and M. Wowogrodzka-Zagorska. 1998. Blood vessels and red blood cells preserved in dinosaur bones. *Annals of Anatomy* 180:73–77.

Pawlicki, R. 1978. Morphological differentiation of the fossil dinosaur bone cells. *Acta Anatomica* 100:411–418.

Pawlicki, R. 1995. Histochemical demonstration of DNA in osteocytes from dinosaur bones. *Folia Histochemica Et Cytobiologica* 33:183–186.

Reisz, R.R., T.D. Huang, E.M. Roberts, S. Peng, C. Sullivan, K. Stein, A.R. LeBlanc, D. Sheih, R. Chang, C. Yang, and S. Zhong. 2013. Embryology of Early Jurassic dinosaur from China with evidence of preserved organic remains. *Nature* 496:210–214.

San Antonio, J.D., M.H. Schweitzer, S.T. Jensen, R. Kalluri, M. Buckley, J.P.R.O. Orgel. 2011. Dinosaur peptides suggest mechanisms of protein survival. *PloS ONE* 6(6): e20381.

Schweitzer, M. H., J.L. Wittmeyer, J.R. Horner, and J.K Toporske. 2005. Soft-tissue vessels and cellular preservation in *Tyrannosaurus rex*. *Science* 307:1952.

Schweitzer, M.H., W. Zheng, C.L. Organ, R. Avci, Z. Suo, L.M. Freimark, V.S. Lebleu, M.B. Duncan, M.G. Vander Heiden, J.M. Neveu, W.S. Lane, J.S. Cottrell, J.R. Horner, L.C. Cantley, R. Kalluri, and J.M. Asara. 2009. Biomolecular characterization and protein sequences of the campanian Hadrosaur B. Canadensis. *Science* 324:626–631.

Schweitzer, M.H., W. Zheng, T.P. Cleland, and M. Bern. 2013a. Molecular analysis of dinosaur osteocytes support the presence of endogenous molecules. *Bone* 52(1): 414–423.

Schweitzer, M.H., W. Zheng, T.P. Cleland, M.B. Goodwin, E. Boatman, E. Theil, M.A. Marcus, and S.C. Fakra. 2013b. A role for iron and oxygen chemistry in preserving soft tissues, cells and molecules from deep time. *Proceedings of the Royal Society B* 281: 20132741. http://rspb.royalsocietypublishing.org/content/281/1775/20132741.

Siek, T.J. 2010. Anceint DNA? *Creation Matters* 15(3): 7.

Snelling, A. 2005. Radiohalos in granites: evidence for accelerated nuclear decay. In Vardiman, L., A.A. Snelling, and E.F. Chaffin (editors), *Radioisotopes and the Age of the Earth, Volume II*, pp. 101–207. Institute for Creation Research, Santee, CA.

Snelling, A.A. 2008. Radiocarbon ages for fossil ammonites and wood in cretaceous strata near Redding, California. *Answers Research Journal* 1:123–144.

Thomas, B., and J. Tomkins. 2014. How reliable are genomes from ancient DNA? *Journal of Creation* 28(3): 92–98.

Thomas, B. 2013. A review of original tissue fossils and their age implications. In Horstmeyer, M. (editor), *Proceedings of the Seventh International Conference on Creationism*. Creation Science Fellowship, Pittsburgh, PA.

Towe, K.M., and A. Urbanek. 1972. Collagen-like structures in Ordovician Graptolite Periderm. *Nature* 237:443–445.

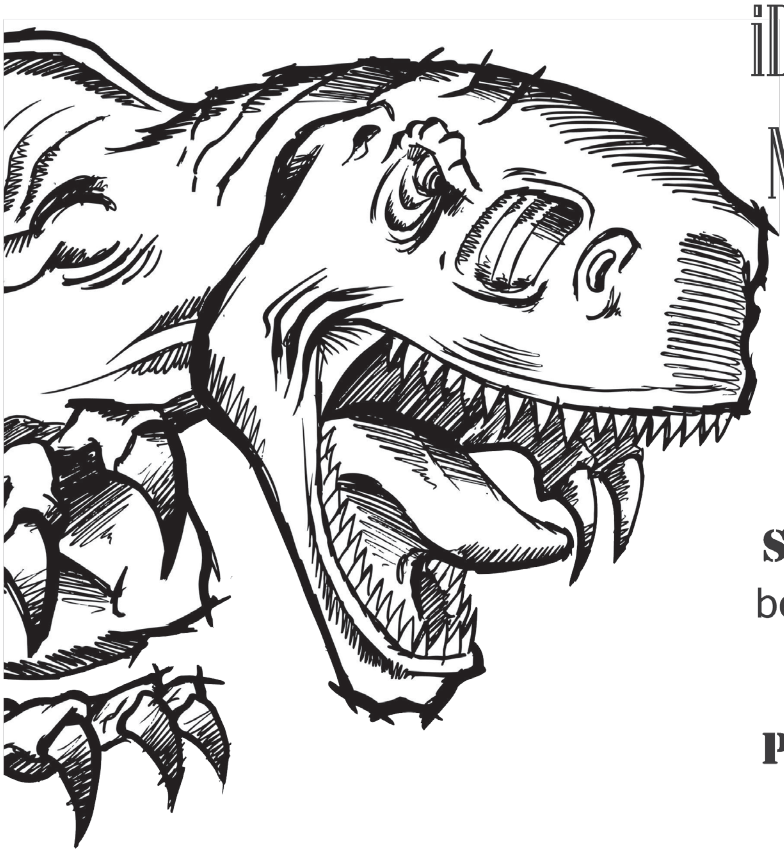
Woodward, S.R., N.J. Weyand, and M. Bunnell. 1994. DNA sequence from Cretaceous Period bone fragments. *Science* 266:1229–1232.

Wyckoff, R.W.G. 1980. Collagen in fossil bones. In Hare, P.E., T.C. Hoering, and K. King (editors), *Biogeochemistry of Amino Acids*, pp. 17–22. Wiley Press, New York, NY.

Zhang, Z., J. Han, X Zhang, J. Liu, and D. Shu. 2004. Soft–tissue preservation in the Lower Cambrian linguloid brachiopod from South China. *Acta Palaeontologica Polonica* 49(2): 259–266.

Zhao, F., D.J. Bottjer, S. Hu, Z. Yin, and M. Zhu. 2013. Complexity and diversity of eyes in Early Cambrian ecosystems. *Scientific Reports* 3:2751.

iDino II



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Soft Bone Material from a Brow Horn of a *Triceratops horridus* from Hell Creek Formation, Montana

Mark Armitage

Abstract

Soft fibrillar bone tissues were discovered within a brow horn of *Triceratops horridus* collected at the Hell Creek Formation in Montana. Soft material was present in pre- and post-decalcified bone. Horn material yielded numerous small sheets of bone matrix that had yet to turn into hard bone. This matrix possessed visible structures consistent with bone osteocytes. Some sheets of soft tissue had multiple layers of intact osteocyte tissues featuring elegant filipodial interconnections and secondary branching. Both oblate and stellate types of osteocyte-like cells were present in sheets of soft tissues. SEM analysis yielded osteocyte cells featuring filipodial extensions of 18 to 20 microns in length. Filipodial extensions were delicate and showed no evidence of any permineralization or crystallization artifact and therefore were interpreted to be soft. This work is the first to report soft tissues from adult *Triceratops* horn in a Creation journal.

Introduction

Many studies have reported the presence of soft tissues and cells in what were thought to be fossilized dinosaur bones from many taxa, including *Tarbosaurus bataar*, *Tyrannosaurus rex*, *Brachylophosaurus canadensis*, and *Triceratops horridus* (Pawlicki, 1978; Pawlicki and Nowogrodzka-Zagorska, 1998; Schweitzer and Horner, 1999; Armitage, 2001; Zylberberg and Laurin, 2011), as well as other extinct organisms such as certain marine turtles (Cadena and Schweitzer, 2012).

Studies using light and electron microscopy have identified tissue components of dinosaur remains, such as red blood cells, endothelial cells, osteocytes, and collagen fibers (Schweitzer et al. 2005, 2007a, 2009). Additionally, molecular peptides and proteins also have been identified and have helped to confirm the cellular nature of these fine structures (Schweitzer et al., 2007b, 2009; Lindgren et al., 2011; San Antonio et al., 2011).

Critiques of these findings have been mounted by Kaye et al. (2008), who contend that these soft remains are merely the leftover biofilms of microbial activity on decaying tissues; however recent analyses seem to confirm that original soft tissues and possibly original molecules do exist in incompletely fossilized remains of extinct animals, including dinosaurs (Schweitzer et al.,

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2009; Lindgren et al., 2011; Cadena and Schweitzer, 2012; Schweitzer et al., 2013).

Furthermore, a large variety of specimens yielding soft tissues supports the fact that soft tissue is not limited to known fossil sites or fossil species. Therefore, recent work has focused on sampling fossils from various types of ancient organisms (dinosaur and otherwise), depositional environments, and geologic time frames to determine the extent of soft tissue presence in Devonian, Triassic, and Cretaceous layers. Comparisons have then been made with recently excavated specimens (Schweitzer et al., 2007a; Zylberberg and Laurin, 2011).

The purpose of this study was to perform microscopy on fresh fossil specimens of *Triceratops* brow horn and ribs for the presence of soft tissues and to characterize any soft tissues found.

Materials and Methods

An intact *Triceratops* horn (HCTH-00) was recovered on May 12, 2012, from a well-sorted, water-buried sandstone within the Hell Creek Formation at a previously unexcavated site in Glendive, Montana. The recovered horn was uncovered, protected with casting materials, and removed. The size and external morphology of the fossil was consistent with other *Triceratops* horns recovered from the Hell Creek Formation.

Disarticulated *Triceratops* ribs (HCTR-11) and vertebrae (HCTV-22) found within a mile of the horn also were recovered for analysis.

In the laboratory, hand-sized pieces of HCTH-00 were fixed in 2.5% glutaraldehyde, buffered with 0.1M sodium cacodylate buffer, rinsed in distilled water and buffer, and stored in PBS. Individual pieces of roughly 20 cm^2 were removed by pressure fracture (HCTH-01, 02, 03) and examined under a dissecting microscope to identify and collect soft material.

Soft materials recovered were washed in pure water and stored in PBS to await further analysis. Other horn specimens (HCTH-04, 05) were processed through a decalcification protocol.

Several pieces about 20 to 50 cm in size were rinsed in water after fixation and were placed into a solution of 14% sodium EDTA at room temperature. EDTA was exchanged every two to four days for a period of four weeks. Significant bone mineral remained after 4 weeks; therefore, some pieces were left in decalcification solution for a period of two months. Complete decalcification never took place; that is, some large fragments of horn remained after two months of soaking, so it is unknown if longer-term soaking would yield soft and transparent, vessel-like tissues such as previously reported (Schweitzer, et al., 2005, 2007a, 2007b, 2009; Lindgren et al., 2011).

Decalcified bone pieces were air-dried, glued to aluminum stubs, sputter coated with gold for 60 seconds at 20mA, and imaged at 20kV on a Hitachi S2500 scanning electron microscope (SEM).

Large strips of thin, light brown, soft material (20cm by 10cm) were recovered from innermost interior sections of other fixed and unfixed, non-decalcified horn bone pieces. Strips were postfixed in 2% osmium tetroxide, dehydrated in a graded series of acetone, and infiltrated with a polymer resin (EMBed-812, Electron Microscopy Sciences, Ft. Washington, PA).

Other strips of soft material were cut into 2cm by 2mm pieces, embedded in OCT (Sakura Tissue Tek, Japan) and sectioned on a Microm (Thermo Scientific) HM550 cryostat at -23C. Cryosections of 9–11 micron thickness were affixed to glass slides, and coverslipped for light microscopy. Photo documentation was performed using a Jenoptik (Jena, Germany) C14+ camera on a Nikon 80i microscope.

Triceratops rib specimens were allowed to air-dry and were subsequently fractured with mechanical pressure yielding 5–10mm-sized pieces suitable for SEM. Pieces of rib with their internal surfaces exposed were sputter-coated and examined with SEM.

Picrosirius stain was employed for the presence of collagen; however, results were inconclusive.

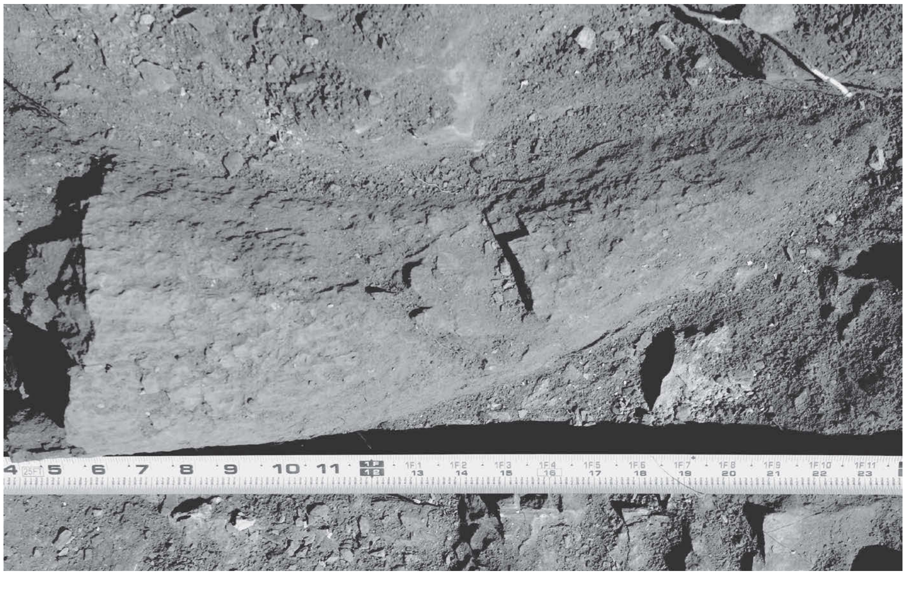


Figure 1. *Triceratops* supraorbital horn specimen, in matrix prior to coating in methylmethacrylate, Hell Creek Formation, Glendive, MT.

Results

A fragment of the *Triceratops* horn (Figure 1) approximately 100 cm long, 22 cm in diameter, and 9 kg in mass was obtained for analysis. The outer sheath of keratin was not found. The horn had been partially buried under 30 cm of homogeneous but loosely packed sandstone and rock. The rock required fracturing by hammer and chisel to free the distal part of the horn. Rib fragments (Figure 2), located separately from the horn, were approximately 15 cm long and had no visible moisture when removed.

Horn material was not completely desiccated but appeared somewhat moist during excavation (Figure 3). Soft, moist, muddy material could be seen surrounding pores of bone vessels on inner horn surfaces (Figure 4).

After removal, the horn fractured into several large pieces (Figures 3, 4). Individual large pieces were wrapped separately in aluminum foil, sealed in containers and transported to the lab for analysis.

Fixed horn material did not seem as friable as unfixed material, possibly because interior soft tissues were stabilized by fixatives. Small (2–5 mm) red and brown plant roots were loosely attached to exterior surfaces and extended into fractures in the horn (see left side of bone in Figure 4).

Pieces of small, fixed material from the inner core of the horn came apart with moderate hand pressure and were found to contain thin, elastic, reddish-brown flaps of soft material (Figure 5, black arrow), which could be peeled away from the bone in sheets and stretched to almost double the original size.

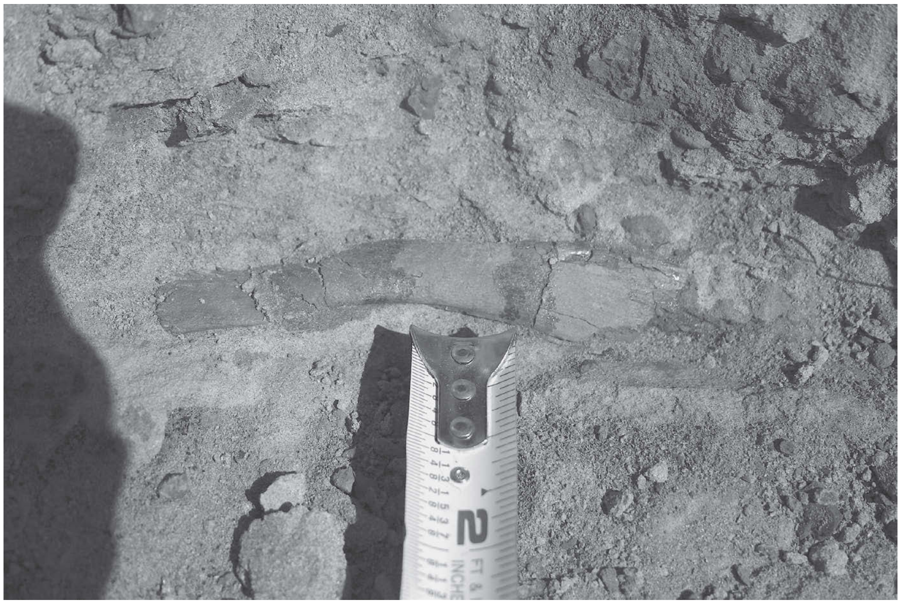


Figure 2. *Triceratops* rib specimen, pre-extraction, Hell Creek Formation, MT.

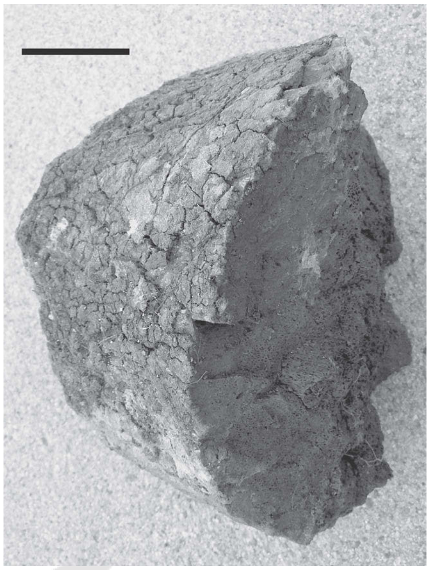


Figure 3. *Triceratops* supraorbital horn, 20 cm by 20 cm unfixed specimen. Scale bar = 2.5 cm.

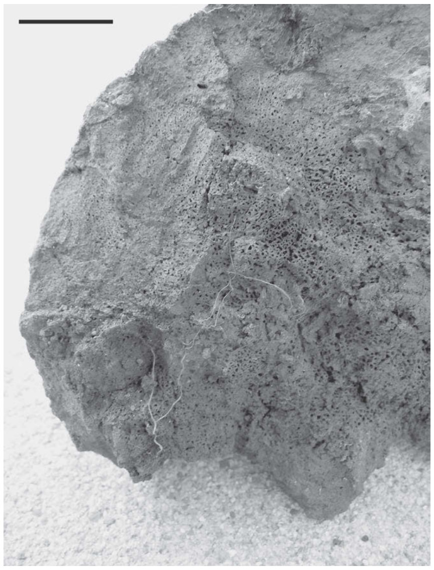


Figure 4. Close-up of bottom surface of horn from Figure 3. Note porosity of vascular channels in horn bone. Scale bar = 1.8 cm.

It was initially thought that this soft material might represent the remains of a biofilm and/or plant material due to the many tiny plant roots associated with it (Figure 6, black arrows). These reddish-brown flaps of soft material and the off-white to milky-white pieces of soft material (Figure 7, white arrows) collected from pre- and post-decalcified bones yielded innumerable small sheets of unmineralized bone matrix with clearly visible cells consistent with lamellar bone osteocytes. Osteocytes also exhibited internal structures consistent with cellular organelles (Figures 9–10, arrows).

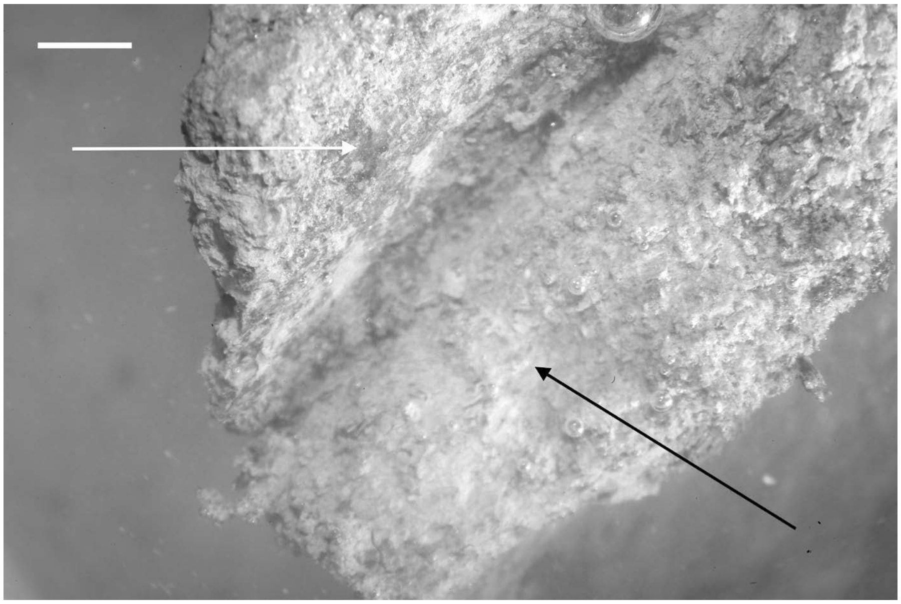


Figure 5. Light micrograph, flap of fixed soft tissue (black arrow) slightly peeled away from undecalcified *Triceratops* bone specimen (white arrow), 10X magnification. Scale bar = 0.75 mm.

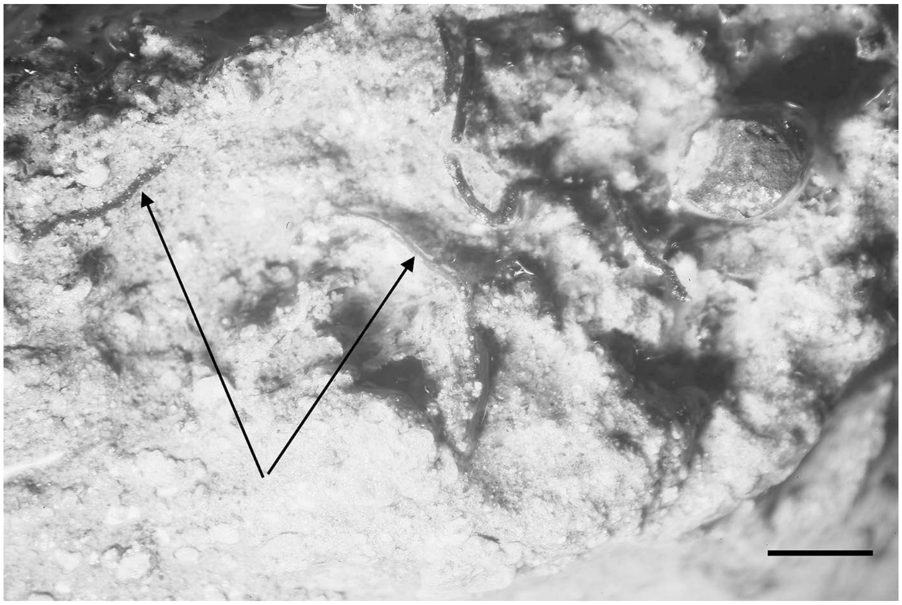


Figure 6. Light micrograph, underside of soft tissue from Figure 5. Note slender, curved plant rootlets (black arrows). Scale bar = 0.5 mm.

The matrix of soft bone densely populated with osteocytes (Figures 8–10) was identical to osteocytes found in compact bone of *Tyrannosaurus rex* femur (Schweitzer et al., 2005, 2013), *Brachylophosaurus canadensis* femur (Schweitzer et al., 2009), and *Prognathodon* sp. femur (Lindgren et al., 2011).

Flexible vessels were not present, and demineralization over two months did not completely dissolve the bone mineral; therefore it was halted. Both processed and unprocessed horn specimens exhibited many clear to milky-white or reddish brown pieces of soft material, which swayed gently upon bone surfaces when solutions were disturbed.

Soft material processed for polymer thin sectioning disintegrated during sectioning, possibly because of a negative effect of the dehydration or maybe because of incomplete infiltration.

Fractured rib specimens contained well-preserved Haversian systems, with many visible lamellae and lacunae (Figure 11). The Haversian canals sometimes were filled with many spherical objects (Figure 12), which are consistent with the size and shape of red blood cells (RBCs). So many RBCs were present that vessel structure was often obscured.

Many soft, hollow, cylindrical tubes were seen to project from Haversian canals (Figure 13) and are consistent with the size and shape of blood vessels.

Non-decalcified horn specimens showed good preservation of mineralized vessel structures surrounded by dense cortical bone. Decalcified horn specimens also were characterized by vessels present in vertical conformation (as they would be in Haversian canals), and interconnecting Volkmann's canals are evident (white arrows, Figure 14), yet all vessel-like structures studied were fully permineralized.

A fractured end of a vessel in Figure 15 (white arrow) is further magnified in Figure 16, and it is clear that microstructures within it (possibly blood and lymph) are also fully permineralized. It therefore appeared that permineralization was selective or, at the least, that some soft tissues were sequestered from the process of fossilization. Further study of the horn must be done to determine if

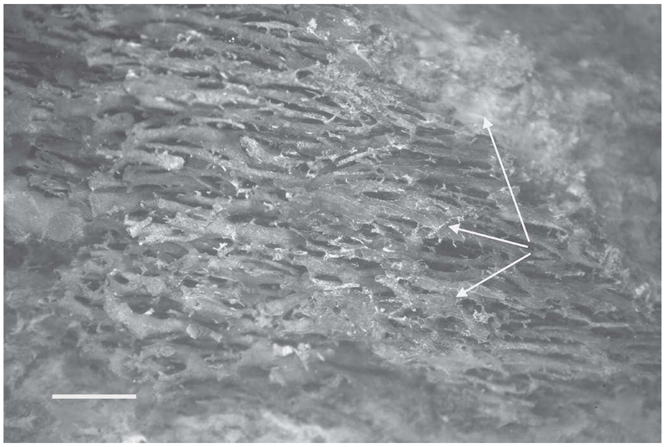


Figure 7. Light micrograph of decalcified portion of *Triceratops* horn bone, 20X magnification. Note white portions of unfossilized, soft tissues that adhere to permineralized vessel elements (arrows). Scale bar = 0.5 mm.

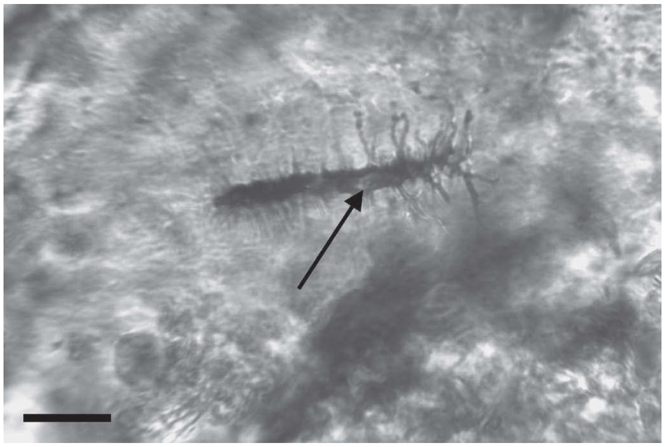


Figure 9. Light micrograph of a section from a sheet of soft, fibrillar bone material from un-decalcified *Triceratops* horn, magnification 1100X. Note intracellular organelle-like structures (arrow). Scale bar = 50 microns.

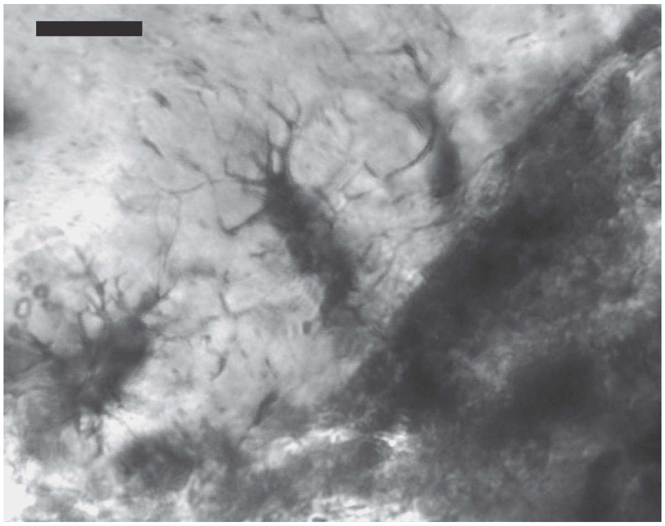


Figure 8. Light micrograph of a section from a sheet of soft, fibrillar bone material from un-decalcified *Triceratops* horn, magnification 800X. Note three osteocytes with fine filopodial processes that interconnect (lower right). Scale bar = 50 microns.

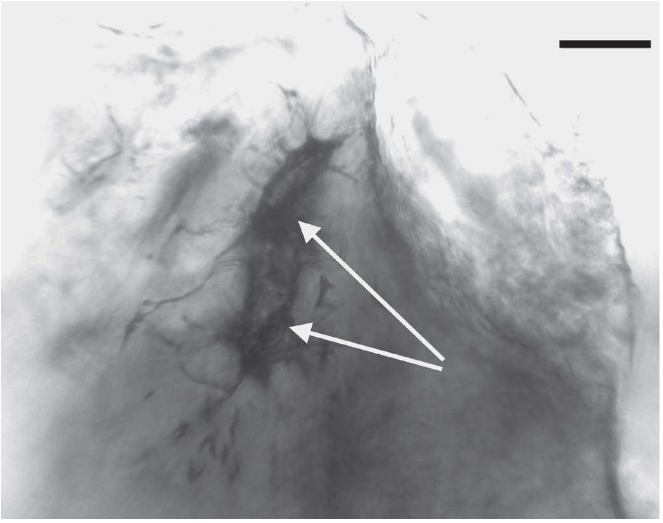


Figure 10. Light micrograph of a section from a sheet of soft, fibrillar bone material from un-decalcified *Triceratops* horn. Note intracellular organelle-like structures (arrows).

this seemingly selective permineralization is related to anatomical differences within the horncore (Happ, 2010).

SEM analysis of decalcified portions of horn shows a large number of osteocytes with very fine filipodial projections lying along the same layer of fibrillar bone (Figures 17–19).

Aliquots of post-decalcification EDTA pipetted onto glass slides were observed to contain individual osteocytes; therefore, small volumes of decalcification solutions collected from the bottom surface of decalcification jars were dialyzed in pure water over several changes, and washed cells were isolated on heat-fixed slides (Figures 20–21).

Discussion

The Hell Creek Formation is a well-characterized and studied rock unit that was first described in the early 1900s (Brown, 1907). It is exposed by the well-known Cedar Creek Anticline at Glendive, Montana and encompasses nearly 700km (Johnson

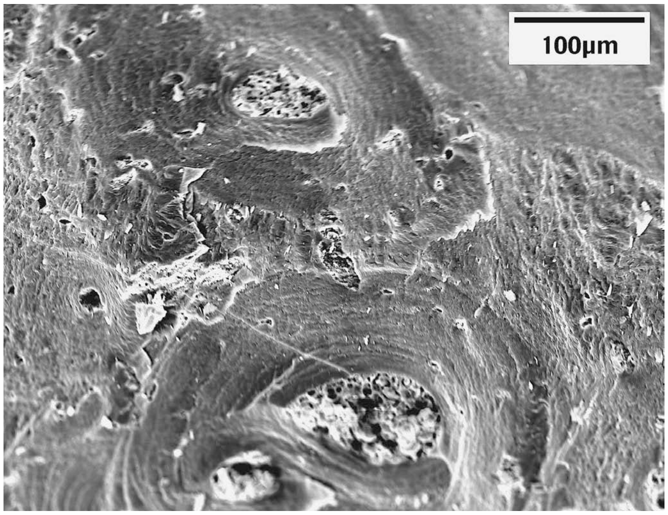


Figure 11. Scanning electron micrograph (SEM) of compact fractured bone material from un-decalcified *Triceratops* rib, magnification 120X. Note well-defined circular Haversian system. Also note that the center of each Haversian system is populated by possible preserved blood products.

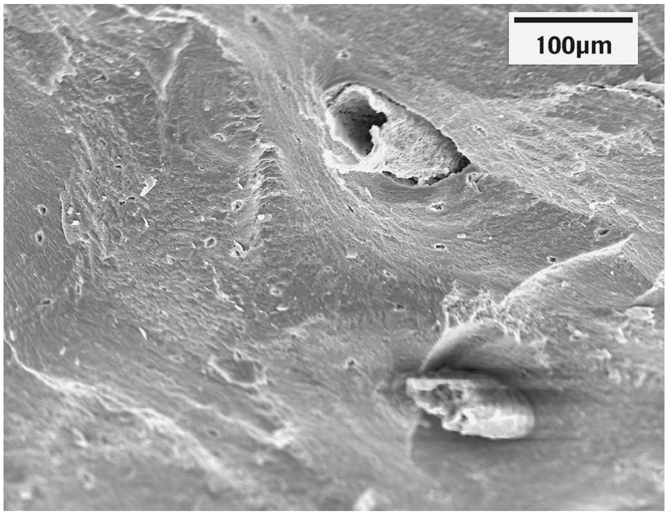


Figure 13. SEM of compact fractured bone material from un-decalcified *Triceratops* rib, magnification 350X. Note hollow blood vessel emanating from Haversian canal. Blood vessels are flexible and pliable.

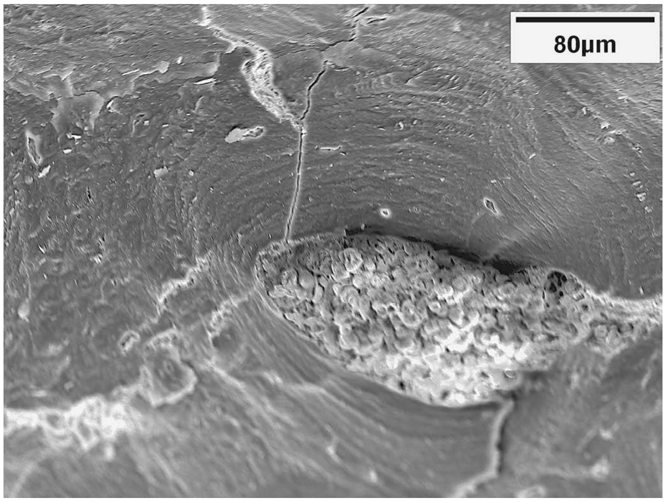


Figure 12. SEM of compact fractured bone material from un-decalcified *Triceratops* rib, magnification 300X. Note well-defined circular Haversian system populated at the center with possible preserved blood products and red blood cell-like microstructures.

et al., 2002). Many valuable fossils have been recovered from the Hell Creek Formation exposed at Glendive, and *Triceratops* remains (including brow horns) are frequently found at that location (Horner, 2001).

This work represents the first to report soft tissues from a *Triceratops* horn (Armitage and Anderson, 2013). This offers a unique opportunity to understand horn form and function. *Triceratops* horn anatomy has been rarely studied, thus much remains to be known about their structure.

Anatomically, *Triceratops* horn offers a unique fossil structure with differentiated inner layers of varying thickness and porosity. Below the keratin sheath (altered and mineralized by the fossilization process) lies an outer bone layer (OBN) of approximately 1–5 mm thickness, composed of compact Haversian bone that is deeply embedded with vascular tissues (Happ, 2010). Within the OBN is the horncore made up of highly vascularized trabecular bone. The trabecular horncore extends almost the entire length of the horn but terminates into a cornusal sinus at the base of the horn where it joins the skull.

Triceratops fossils are considered common within the Hell Creek Formation (Happ, 2010). Discovery of soft tissue in *Triceratops* horn provides additional insight into the nature of fossilization and extends our understanding on the prevalence of preserved original dinosaur tissue.

No tissues or vessels floated away from bones in solution. As described, however, solutions were examined post-decalcification for the presence of free-floating osteocytes, and they were successfully isolated. It is hoped that planned immunohistochemistry experiments will detect endogenous proteins.

Decalcification was performed for 20 weeks, which would have seemed sufficient time to expose soft tissues for collection, but bone mineral was never fully dissolved, so it is unknown if soft vessels would have remained had decalcification been carried out longer. Further study is required to determine if

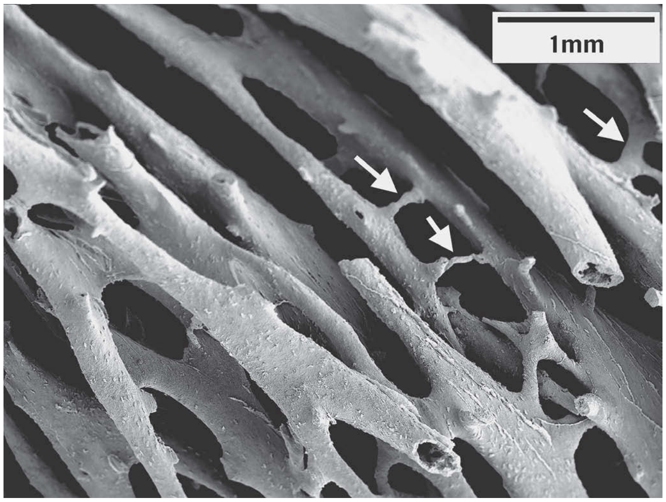


Figure 14. SEM of decalcified bone material from highly vascular portion of *Triceratops* horn, magnification 25X. All of the bone mineral has been dissolved away, leaving intact permineralized vessels. Volkmann's canals link Haversian canal vessels together (white arrows).

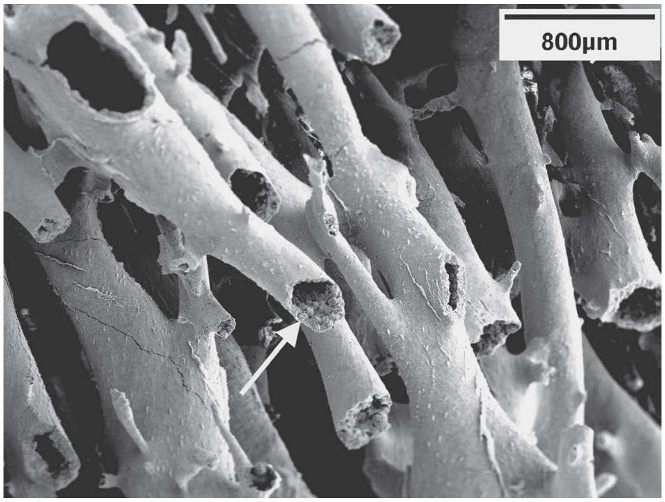


Figure 15. SEM close-up similar to Figure 14. Magnification 30X. Note possible blood products lining inner wall of hardened vessel (white arrow).

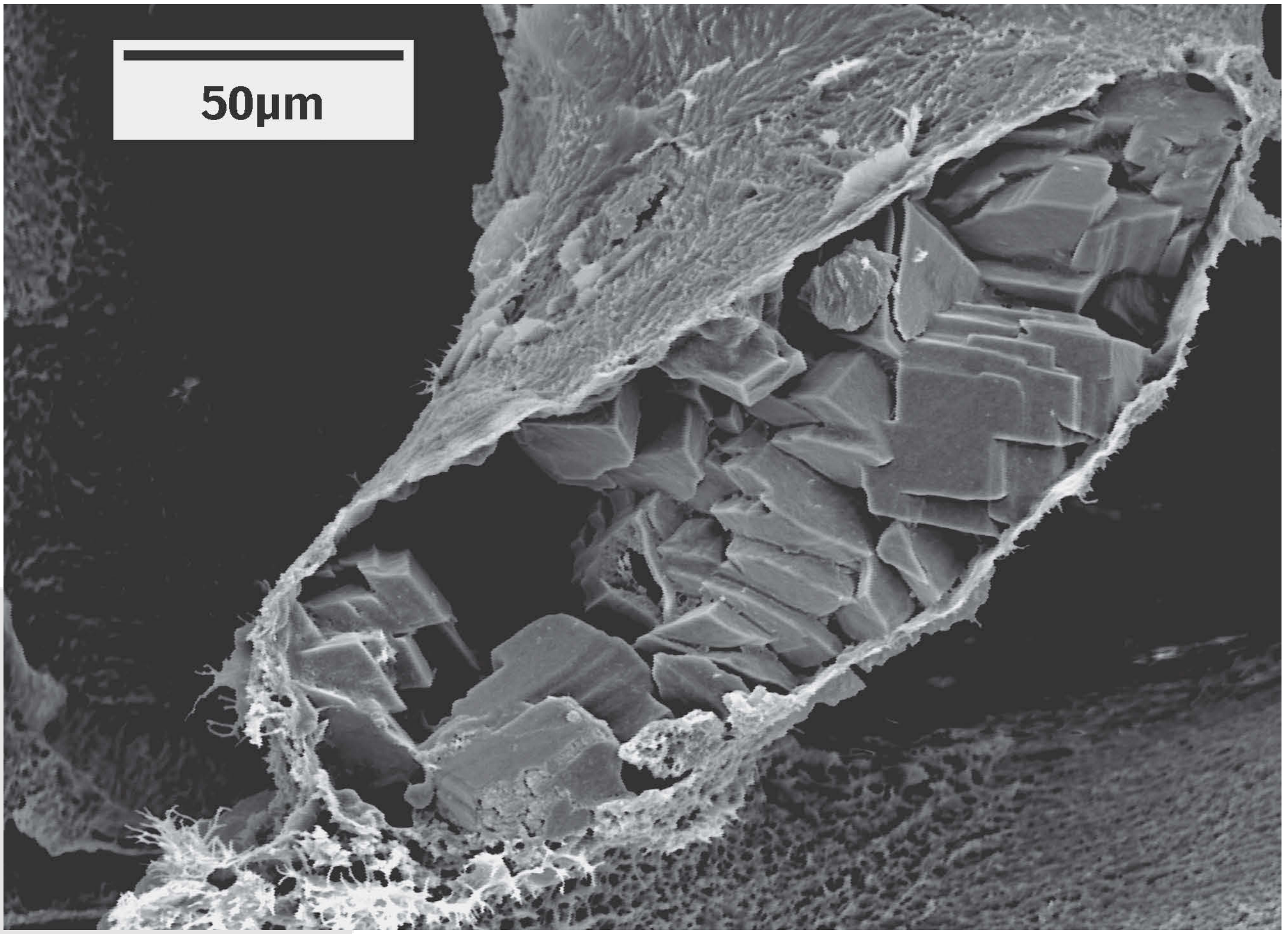


Figure 16. SEM close-up from another vessel. Magnification 200X. Note crystallized nature of possible blood products lining inner wall of hardened vessel.

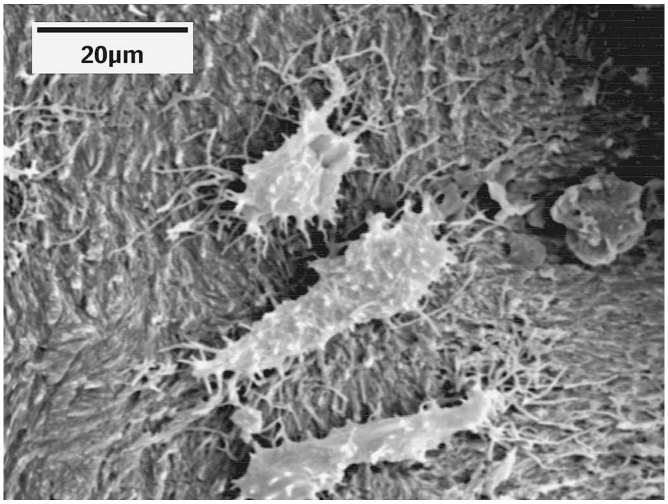
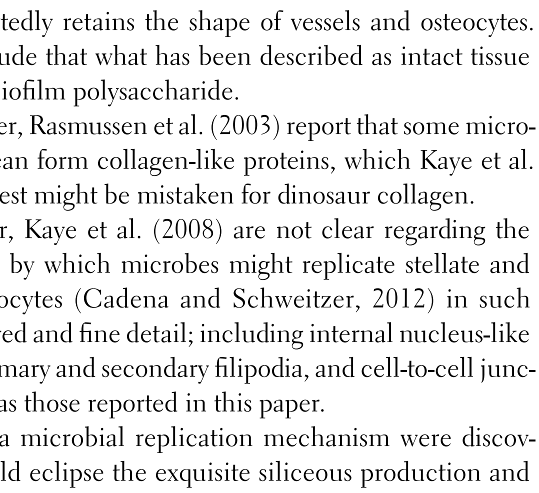


Figure 17. SEM of decalcified bone material from *Triceratops* horn, magnification 200X. Note two large oblate osteocytes lying on fibrillar bone matrix.



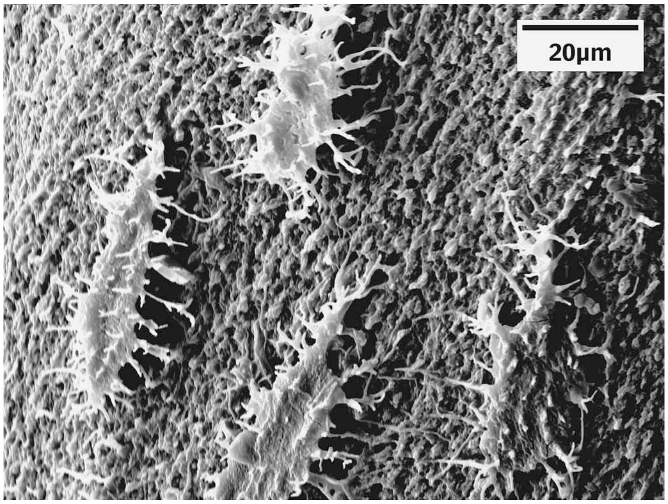


Figure 19. SEM of decalcified bone material from *Triceratops* horn, magnification 1000X. Note four osteocytes lying on fibrillar bone matrix. Cell filipodia are anchored into the bone matrix and have diameters approaching 200nm.

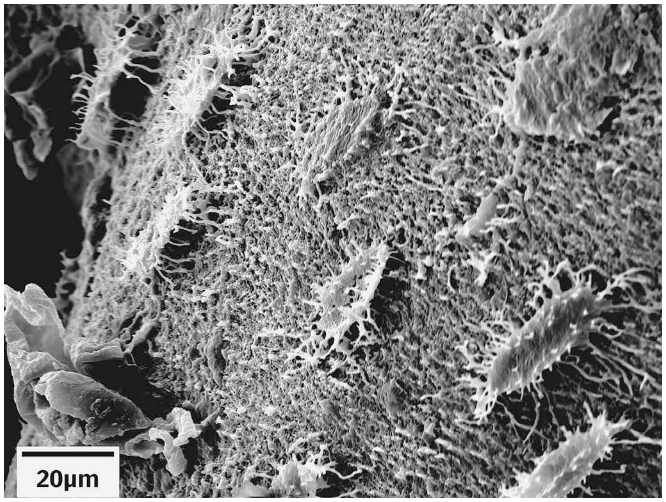


Figure 18. SEM of decalcified bone material from *Triceratops* horn, magnification 600X. Note oblate osteocytes lying on fibrillar bone matrix. Tiny white filipodial processes from cells beneath the layer can be seen extruding through.

intact, soft vessels might be recovered from completely demineralized horn specimens.

Kaye et al. (2008) maintain that the soft tissue from dinosaur fossils is polysaccharide from a microbial biofilm. They suggest that the polysaccharide film forms a cast of the tissue while bacteria are decomposing organic remains (soft bone tissues). Once dissolved from the fossilized bone matrix, this film purportedly retains the shape of vessels and osteocytes. They conclude that what has been described as intact tissue is actually biofilm polysaccharide.

Moreover, Rasmussen et al. (2003) report that some microorganisms can form collagen-like proteins, which Kaye et al. (2008) suggest might be mistaken for dinosaur collagen.

However, Kaye et al. (2008) are not clear regarding the mechanism by which microbes might replicate stellate and oblate osteocytes (Cadena and Schweitzer, 2012) in such well-preserved and fine detail; including internal nucleus-like spheres, primary and secondary filipodia, and cell-to-cell junctions, such as those reported in this paper.

If such a microbial replication mechanism were discovered, it could eclipse the exquisite siliceous production and assembly systems employed by diatom cells to create the compelling geometric valves known by microscopists the world over (Sumper and Bruner, 2006; Tesson and Hillebrand, 2010). Sophisticated replication systems used by diatoms are very poorly understood, but to uncover the method by which a bacterial biofilm could replicate the very cells the bacteria are decomposing would be astonishing.

In addition, it is not clear how such biofilm structures could themselves survive the ravages of time. Once produced, other microorganisms could begin to digest these initial biofilms. Such biofilm regeneration over time would not be consistent with the retention of the cellular detail reported here and by others.

Bone material examined in this study was found undesiccated; therefore, microbial activity could have been supported

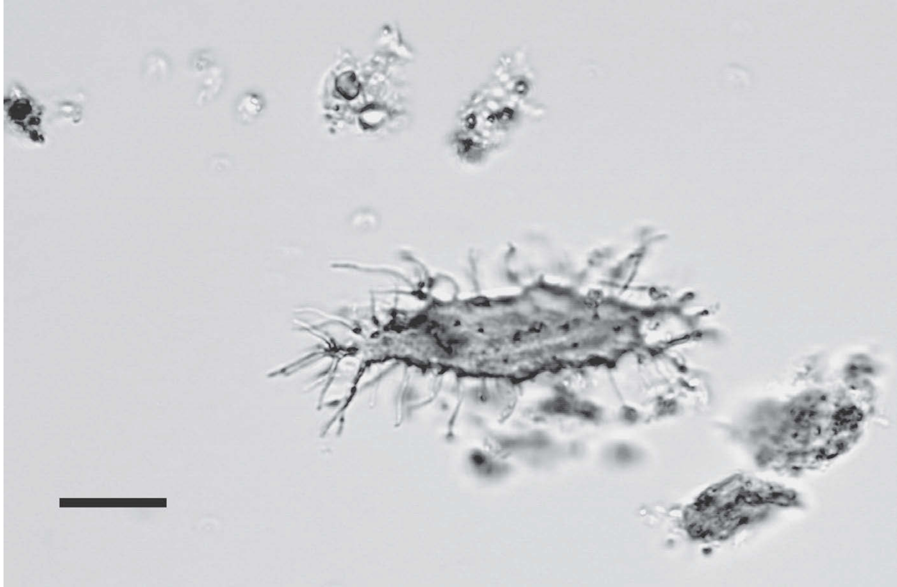


Figure 20. Light micrograph of individual isolated osteocyte on a glass slide.

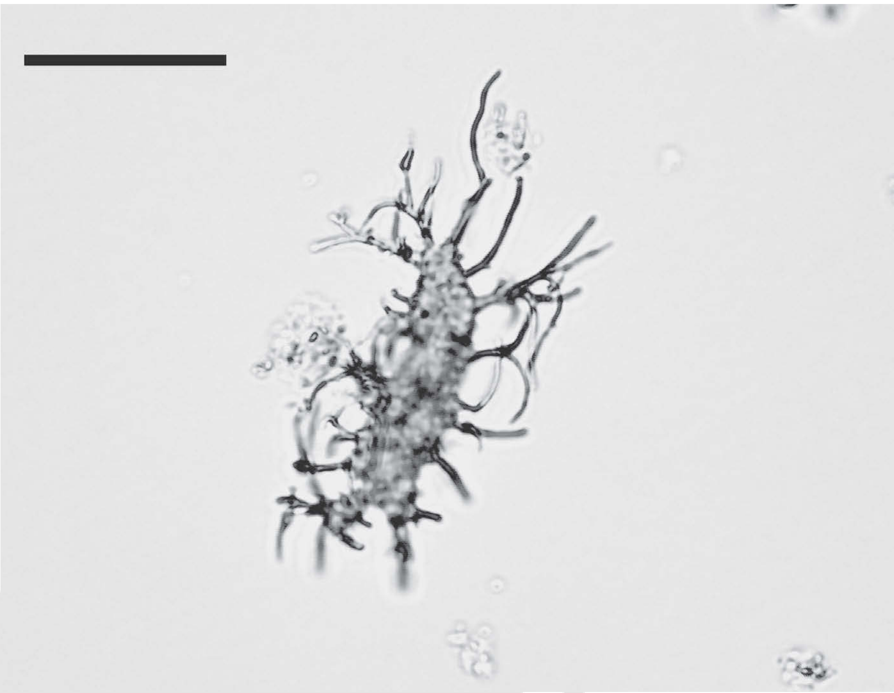


Figure 21. Higher magnification light micrograph of a single decalcified osteocyte.

within this specimen after burial.

The most parsimonious explanation is that these are original tissues, not highly reproducible organic ghost images of original tissues.

The fact that any soft tissues were present in this heavily fossilized horn specimen would suggest a selective fossilization process, or a sequestration of certain deep tissues as a result of the deep mineralization of the outer dinosaur bone, as described by Schweitzer et al. (2007a).

As mentioned previously, however, the horn was not desiccated when recovered and actually had a muddy matrix deeply embedded within it, which became evident when the horn fractured.

Additionally, in the selected pieces of this horn that were processed, soft tissues seemed to be restricted to narrow slivers or voids within the highly vascular bone, but further work is needed to fully characterize those portions of the horn that contained soft material.

It is unclear why these narrow areas resisted permineralization and retained a soft and pliable nature. Nevertheless, it is apparent that certain areas of the horn were only lightly impacted by the degradation that accompanied infiltration by matrix and microbial activity.

Proposing that these elastic sheets of reddish-brown soft tissues are microbial biofilm structures is not consistent with our observations of fine structure of osteocyte filipodia and their internal microstructures resembling cellular organelles (Figures 9–10).

Nevertheless the question remains: How could microbes have replicated the fine detail visible in these structures?

In addition, the filipodial processes show no evidence of crystallization, as do the fractured vessels in Figure 16, and some filipodial processes taper elegantly to 300nm widths and smaller (Figure 19).

Furthermore, if biofilms represent the sole component of these soft tissues, as suggested by Kaye et al. (2008), why are they found only within compact bone? Certainly there would seem to be sufficient nutrients in the matrix surrounding buried bones to support the production of additional biofilms. Moreover, why are the decaying roots that no longer support plant functions not covered over or completely replicated themselves by biofilms? Finally, biofilm production must have taken place within years or decades after burial in order to capitalize on nutrients available in their original form and to faithfully replicate ultrastructure before autolysis. It does not seem reasonable to suggest the original tissues would not survive through deep time but replicated structures captured in biofilms would.

We believe it significant that large, intact sheets of dinosaur fibrillar bone matrix seemed to be more densely populated (in the z-axis) with osteocytes than in previous studies of dinosaur material (Figure 8). It also was interesting that *Triceratops* soft tissues (particularly those containing multiple layers of osteocytes) did not require staining for light microscopy.

Osteocytes normally display very fine structure, including nucleus-like spheres and secondary branching of filipodia in freshly sectioned material. We observed these nucleus-like spheres and secondary branching of filopodia.

Similarly, many transparent osteocytes and nucleus-like structures were observed in the *T. rex* material examined by Schweitzer et al. (2005, 2007b, 2013). We also observed transparent tissue structures. Initially it was thought that simply empty lacunae were being imaged; however, SEM analysis verified the presence of oblate and stellate osteocytes (Cadena and Schweitzer, 2012) with very fine structural details (to within 300nm or less). Some filipodial extensions in this study reached 18–20 microns in length, almost twice the length of those previously reported (Figures 18–19). Therefore our data is fully consistent with previous reports, and this extensive detail further complicates a biofilm explanation.

Contrary to Kaye et al. (2008), who claimed that these structures are the remains of bacterial biofilms, the vessel-like structures emanating from many of the vascular canal walls were thick, fully cylindrical, and had undulating wall surfaces consistent with extant vessel tissues (Figures 13, 16).

Furthermore, the thin strips of soft material and the vessel-like structures we discovered extended deep into the bone, which is uncharacteristic of superficial biofilms.

Schweitzer et al. (2005, 2007a, 2007b, 2009) and Asara et al. (2007) analyzed different dinosaur fossils and observed osteocytes with original transparency, extensive filipodia, and internal contents (such as a defined nuclei) within the osteocytes. As noted, this is inconsistent with the suggestion that the observed osteocytes are actually biofilm imprints mimicking the morphology of octeocytes. Such imprints would not have defined nuclei or other internal cell structures. In addition, antibodies for avian collagen I exhibited an affinity for collagen isolated from *T. rex* fossils, and this collagen was degraded by modern collagenase (Schweitzer et al., 2005).

Antibodies with an affinity for both avian and reptilian proteins also had affinity for *B. canadensis* (Schweitzer et al., 2009). Bern et al. (2009) further analyzed the specimens used by Asara et al. (2007) and confirmed the presence of an avian-like collagen with no indication of microbial collagen-like proteins. Moreover, Schweitzer et al. (2013) have detected the affinity of an avian collagen-specific antibody and a DNA-specific antibody for molecules in the tissue samples. Such affinity is indicative of the presence of avian collagen and nucleic acids in the dinosaur tissue.

Cumulatively, these characteristics are consistent with the presence of intact tissue and inconsistent with the putative presence of microbial biofilm material. Thus, it is also concluded that these data are fully consistent with the detection of intact cells and tissues that have been preserved in these *Triceratops* fossils.

References

Armitage, M. 2001. Scanning electron microscope study of mummified collagen fibers in fossil *Tyrannosaurus rex* bone. *Creation Research Society Quarterly* 38:61–66.

Armitage, M.H., and K.L. Anderson. 2013. Soft sheets of fibrillar bone from a fossil of the supraorbital horn of the dinosaur *Triceratops horridus*. *Acta Histochemica* 115(6): 603–608.

Asara, J.M., M.H. Schweitzer, L.M. Freimark, M. Phillips, and L.C. Cantley. 2007. Protein sequences from Mastodon and *Tyrannosaurus rex* revealed by mass spectrometry. *Science* 316: 280–285.

Bern, M., B.S. Phinney, and D. Goldberg. 2009. Reanalysis of *Tyrannosaurus rex* mass spectra. *Journal of Proteome Research* 8:4328–4332.

Brown, B. 1907. The Hell Creek beds of the Upper Cretaceous of Montana. *Bulletin of the American Museum of Natural History* 23:823–845.

Cadena, E.A., and M.H. Schweitzer. 2012. Variation in osteocytes morphology vs bone type in turtle shell and their exceptional preservation from the Jurassic to the present. *Bone* 51:614–620.

Happ, J.W. 2010. New evidence regarding the structure and function of the horns in *Triceratops* (Dinosauris: Caratopsidae). In Ryan, M.J., B.J. Chinnery-Allgeier, and D.A. Eberth (editors), *New Perspectives on Horned Dinosaurs*, pp. 271–281. Indiana University Press, Bloomington and Indianapolis, IN.

Horner, J. 2001. *Dinosaurs under the Big Sky*. Mountain Press Publishing Company, Missoula, MT.

Johnson , K.R., D.J. Nichols, and J.H. Hartman. 2002. Hell Creek Formation: a 2001 synthesis. In Hartman, J.H., K.R. Johnson, and D.J. Nichols (editors), *The Hell Creek Formation and the Cretaceous-Tertiary boundary in the northern Great Plains: An integrated continental record of the end of the Cretaceous*, pp. 503–510. Geological Society of America Special Paper 361, Boulder, CO.

Kaye, T.G., G. Gaugler, and Z. Sawlowicz. 2008. Dinosaur soft tissues interpreted as bacterial biofilms. *PLoS One* 3(7): e1–7.

Lindgren, J., P. Uvdal, A. Engdahl, A.H. Lee, C. Alwmark, K.E. Bergquist, E. Nilsson, P. Ekstrom, M. Rasmussen, D.A. Douglas, M.J. Polcyn, and L.L. Jacobs. 2011. Microspectroscopic evidence of Cretaceous bone proteins. *Plos One* 6(4): e1–11.

Pawlicki, R. 1978. Morphological differentiation of the fossil dinosaur bone cells. *Acta Anatomica* 100:411–418.

Pawlicki, R., and M. Nowogrodzka-Zagorska. 1998. Blood vessels and red blood cells preserved in dinosaur bones. *Annals of Anatomy* 180:73–77.

Rasmussen, M., M. Jacobsson, and L. Björck. 2003. Genome-based identification and analysis of collagen-related structural motifs in bacterial and viral proteins. *The Journal of Biological Chemistry* 278:32313–32316.

San Antonio, J., M. Schweitzer, S. Jensen, R. Kalluri, M. Buckley, and J. Orgel. 2011. Dinosaur peptides suggest mechanisms of protein survival. *PLoS One* 6: e20381.

Schweitzer, M.H., and J.R. Horner. 1999. Intravascular microstructures in trabecular bone tissues of *Tyrannosaurus rex*. *Annales de Paléontologie* 85(3): 179–192.

Schweitzer, M.H., J.L. Wittmeyer, J.R. Horner, and J.K. Toporski. 2005. Soft-tissue vessels and cellular preservation in *Tyrannosaurus rex*. *Science* 307:1952–1955.

Schweitzer, M.H., J.L. Wittmeyer, and J.R. Horner. 2007a. Soft tissue and cellular preservation in vertebrate skeletal elements from the Cretaceous to the present. *Proceedings of the Royal Society B* 274:183–197.

Schweitzer, M.H., Z. Suo, R. Avci, J.M. Asara, M.A. Allen, F.T. Arce, and J.R. Horner. 2007b. Analyses of soft tissue from *Tyrannosaurus rex* suggest the presence of protein. *Science* 316:277–280.

Schweitzer, M.H., W. Zheng, C. Organ, R. Avci, Z. Sun, L. Freimark, V. Lebleu, M. Duncan, M. Heiden, J. Neveu, W. Lane, J. Cottrell, J. Horner, L. Cantley, R. Kalluri, and J. Asara. 2009. Biomolecular characterization and protein sequences of the Campanian Hadrosaur *B. Canadensis*. *Science* 324:626–631.

Schweitzer, M.H., W. Zheng, T.P. Cleland, and M. Bern. 2013. Molecular analyses of dinosaur osteocytes support the presence of endogenous molecules. *Bone* 52:414–423.

Sumper, M., and E. Bruner. 2006. Learning from diatoms: nature's tools for the production of nanostructured silica. *Advanced Functional Materials* 16:17–26.

Tesson, B, and M. Hillebrand. 2010. Dynamics of silica cell wall morphogenesis in the diatom *Cyclotella cryptica*: substructure formation and the role of microfilaments. *Journal of Structural Biology* 169:62–74.

Zylberberg, L., and M. Lauren. 2011. Analysis of fossil bone organic matrix by transmission electron microscopy. *Comptes Rendus Palevol* 10:357–366.

Creation Research Society Quarterly 2015. 51:259–267.

Dinosaur Tissue or Bacterial Biofilms?

Kevin Anderson*

Abstract

Pliable soft tissue containing detailed cellular structures has been detected in numerous dinosaur fossils. Studies have also reported extracting and identifying several animal proteins (e.g., collagen and actin) from this tissue. Since predicted decay rates are not consistent with tissue and biomolecules being preserved for millions of years, these findings challenge the assigned ages of the dinosaur fossils. Different explanations have been offered for how tissue could survive for extended periods of time. One explanation is that this tissue is actually a bacterial biofilm with a replica imprint of dinosaur cells and the biomolecules are of bacterial origin. Bacterial biofilms have even been shown to have a significant role in the fossilization processes. However, biofilms have not been shown to replicate the cellular detail found in dinosaur tissue. Also, amino-acid sequence, antibody affinity, and microspectroscopic analysis reveals significant difference between bacterial proteins and those proteins extracted from the soft tissue. Thus there is no substantial evidence that the pliable material extracted from dinosaur fossils is contaminating biofilm.

Introduction

Numerous studies have reported the detection of pliable tissue and even biomolecule remnants (such as protein fragments) in dinosaur fossils (Thomas, 2015). These discoveries have presented evolutionists with a conflicting situation. Based upon the standard (evolution-biased) dating paradigm, dinosaurs lived and died at least 65 million years ago. Yet, the persistence of this pliable tissue and some partially intact biomolecules contradicts decay rates and decomposition studies for biological material (Allentoft et al., 2012; Bada et al., 1999; Buckley et al., 2008). The presence of this tissue indicates these dinosaur fossils are far less than 65 million years of age. In fact, an age of merely a few thousand years would be a much more consistent conclusion.

Thus, evolutionists have sought various alternative explanations for the presence of this tissue (see the discussion of DeMassa and Boudreaux, 2015; and Thomas, 2015). One of these alternatives is that the detected material is not actual preserved original tissue. Rather, it is a bacterial biofilm with general characteristics similar to animal tissue.

Kaye et al. (2008) argue that bacterial "biofilms share a closer molecular make-up" to the detected pliable "soft tissue" material than does actual bone tissue.

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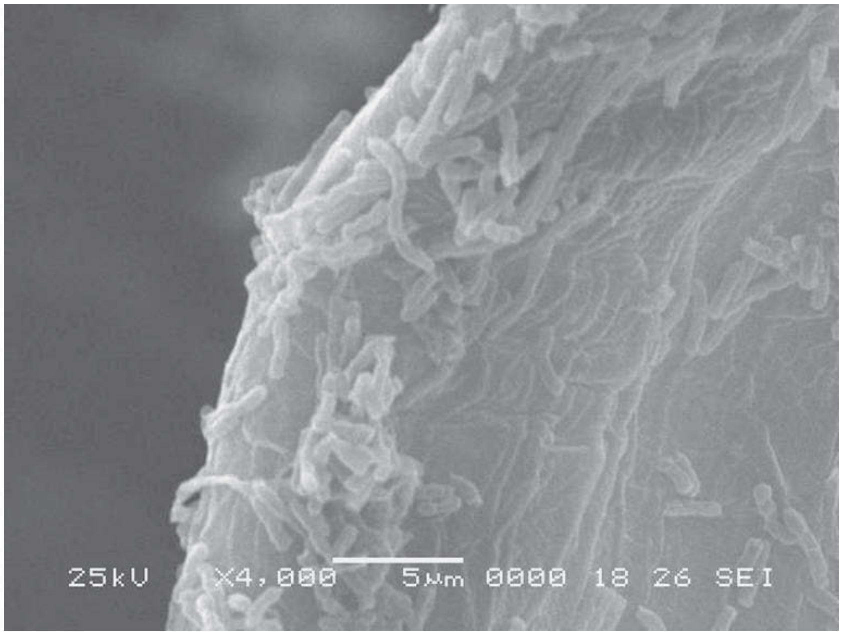

Figure 1. Electron micrograph (4,000x) of bacterial cells attaching to a surface as the first step of biofilm formation.

They further argue that studies, such as Asara et al. (2007), mistakenly conclude that the collagen detected in the fossil was of dinosaur origin. Instead, Kaye et al. (2008) counter that these studies have merely detected a collagen-like protein in the fossils, with little justification to assume this is actual dinosaur collagen. In fact, bacteria have been previously reported to make a collagen-like protein (Rasmussen et al., 2003). Thus, Kaye et al. (2008) suggest that both the pliable tissue and the proteins found in dinosaur specimens are likely not original but of bacterial origin. Many bloggers and other science commentators were quick to accept, or at least promote, this biofilm explanation (e.g., Cambell, 2008; Hecht, 2008; Smith, 2008).

More recently, Raff et al. (2008, 2013, 2014) and Hu et al. (2011) have proposed a mechanism for bacterial involvement in soft-tissue preservation. While not specifically challenging the validity of dinosaur soft-tissue reports, their work reintroduces a role for biofilms. The extent of this role warrants closer examination of the nature of biofilms and the dinosaur soft-tissue data.

Bacterial Biofilms

Most people are familiar with the aromatically fruity slime on forgotten leftovers in the refrigerator.

Bacteria frequently exist in such a microbial community, which can be found attached to a wide variety of surfaces. This community, known as a biofilm, provides a microenvironment that facilitates a synergistic interaction of bacteria within the film. As such, biofilms frequently offer a significant survival advantage for bacteria, which helps explain their common occurrence in virtually all microbial niches.

The initial stage of biofilm development involves a few bacterial cells binding to some type of surface (e.g., teeth, bone, food, tissue, rock) (Figure 1). After binding, the cells begin to replicate and can form an immature biofilm, which is usually small and contains a low number of bacteria (comprised of perhaps only one or a few species). As the biofilm matures, its population size increases, as well as the size and shape of the film (Figure 2). During maturation, the composition of the bacterial population may become very diverse (Rendueles and Ghigo, 2012). Biofilm structures also vary, depending on the nature of the surface on which the biofilm is adhering (Hochbaum and Aizenberg, 2010). Mature biofilms may ultimately be composed of a single species or multiple species, and bacterial interactions also become more complex (Anderson, 2003; Elias and Banin, 2012; Rendueles and Ghigo, 2012).

The growth of the biofilm population is affected by the availability of nutrients, environmental temperature, and the population composition. Cells near the biofilm surface have ready access to nutrients from the environment, while cells in the interior are more dependent upon metabolites from surrounding bacteria for their energy substrates. Thus, different locations in the biofilm will likely be populated by different species of bacteria. This also means that cells within a biofilm are often in a dynamic of movement and metabolism.

As the biofilm matures, the metabolism of the bacteria begins to either increase or decrease the internal pH. The pH change stimulates bacterial production of mineral precipitates (e.g., calcium carbonate and hydroxyapatite) (Cooke et al., 1999; VanGlulck et al., 2003). This situation is frequently encountered in urinary catheters (Morris et al., 1999; Stickler et al., 1993) and leachate collection systems of waste landfills (Cooke et al., 2001), causing encrustation and fouling.

Maturation of biofilms also involves bacterial secretion of a polysaccharide matrix known as an extracellular polymeric substance (EPS). In most

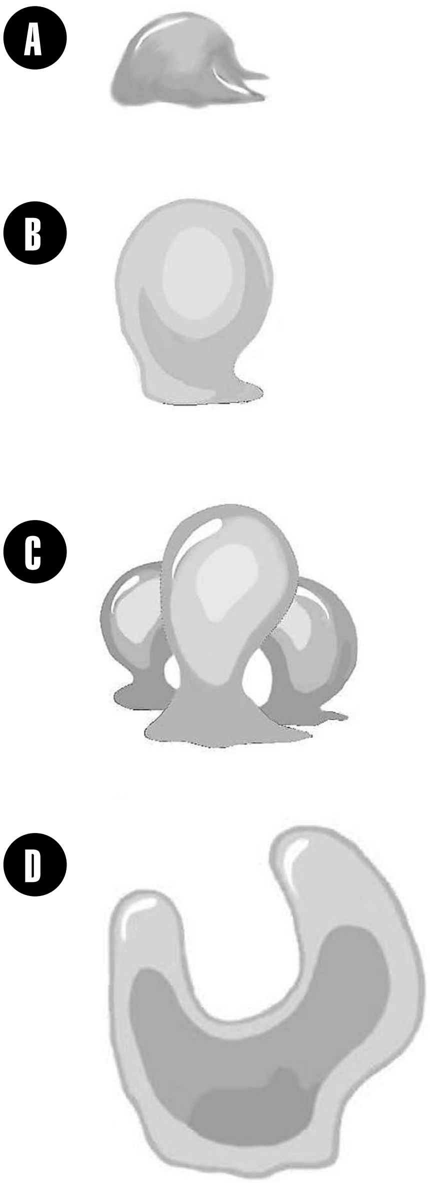

Figure 2 (*left*). Development of bacterial biofilm structures. After initial attachment of cells to a surface, the population size steadily increases and begins production of EPS: (A) early biofilm structure following initial EPS production, (B) continued growth of the biofilm from increased accumulation of EPS, (C) biofilm begins to expand and potentially form multiple structures, and (D) maturation of the biofilm.

biofilms, EPS accounts for 90% of the matrix (Flemming and Windgender, 2010). This EPS serves to provide both a protective layer and a scaffold to hold the bacteria within the community (Flemming and Wingender, 2010). As a hydrogel, EPS consists mostly of polysaccharide (with some proteins, nucleic acids, and sometimes humic substances). The composition of the polysaccharides in the EPS varies depending upon the types of bacteria in the biofilm. EPS typically has properties similar to gums (polysaccharides that greatly increase a solutions viscosity).

Within this EPS matrix, bacterial cells are maintained in close proximity, enabling a variety of microbial interactions (e.g., nutrient sharing, water transport, and chemical protection). In addition, this structure serves as a platform for cell-to-cell communication (including a phenomenon called *quorum sensing*) (Schuster and Markx, 2014). Water columns may also form, enabling water transport to cells in the biofilm interior.

The resulting 3-dimensional EPS matrix can have a variety of shapes and sizes (Figure 3). Comparisons of different biofilms formed by different organisms and strains typically show that many, if not most, biofilms are very simple structures. Biofilm structure and shape is generally a function of population composition and environmental conditions. Typically, biofilms are flat and featureless, or they consist of simple aggregates, especially when nutrient availability is limited (Bridier et al., 2010). Some bacteria can form biofilms with a patchy characteristic, or a more complex pillar or mushroom-shaped structure (Schuster and Markx, 2014). Many biofilms are microscopic in size, but some very mature biofilms may become fairly large and readily visible.

Biofilms appear to form by random growth or simple chaotic aggregation processes. However, even in such simple models, different microenvironments can be found within the biofilm. The presence of EPS limits convective mass transfer of nutrients from the medium to the cells. It also limits movement of metabolic substrates, products, and intermediates within the matrix. Cells usually need a close juxtaposition for cell-to-cell transfer of metabolites or other chemicals. Thus, diffusion is the primary mechanism for transferring nutrients and chemicals in a biofilm, and the viscosity of the EPS causes chemical gradients to form. This tends to favor specific bacterial populations within each gradient, giving a segregation of species within a biofilm.

Biofilm or Tissue?

The relationship of bacterial biofilms and tissue preservation has been studied for several years. Raff et al. (2008) suggest that embryo preservation occurs with a biofilm consuming the embryo and simultaneously forming a replica of the embryo. In a follow-up study, they observe in greater detail the process of how microbial invasion stabilizes the embryo tissue and subsequently replaces the embryo with a three-dimensional biofilm, which mimics the embryo's morphology (Raff et al., 2013). McNamara et al. (2009) also report that detail of a Miocene frog's soft tissue is preserved by a bacterial biofilm replica.

This model for biofilm replica of tissue prompted Kaye et al. (2008) to offer an alternative interpretation of dinosaur soft tissue. They report that microscopic analysis of pliable sheets from dinosaur fossils reveals the presence of bacterial biofilms in the pores of the bone. They interpret this biofilm layer as forming an endocast of the original cell and bone

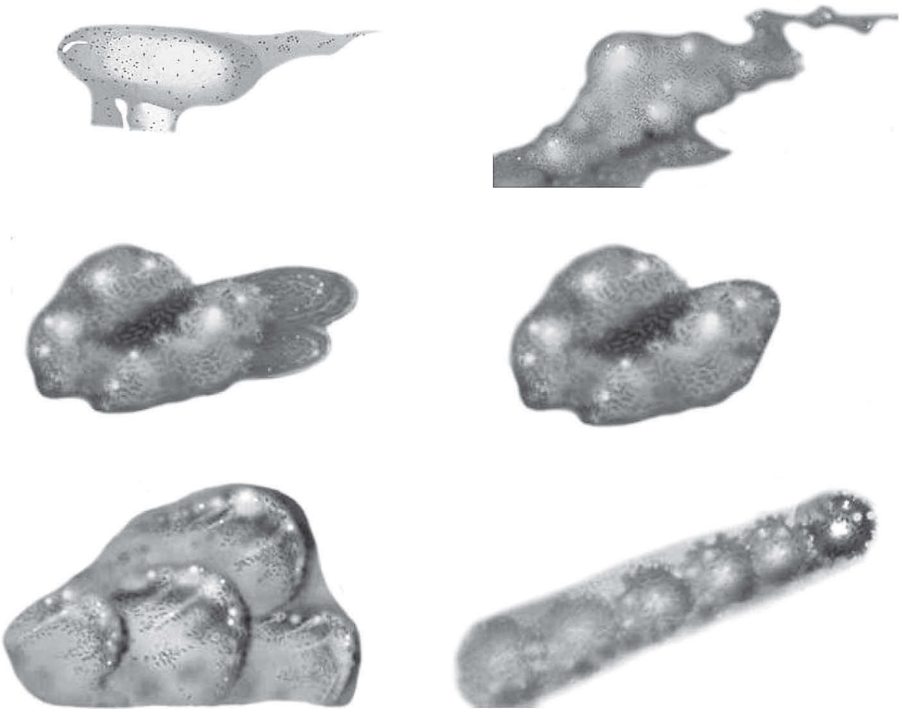

Figure 3. Examples of different mature biofilm structures/morphologies.

matrix. Thus, they suggest that these endocasts have been misinterpreted as blood vessels and pliable tissue containing osteocyte cells. The researchers further offer that the natural pliability of a biofilm enables it to retain the shape of the surface it formed upon, in this case the dinosaur tissue.

They are somewhat vague regarding the mechanism of this biofilm endocast. Presumably, they are suggesting the endocast forms upon the cells' cast, as suggested by Raff et al. (2008, 2013). The biofilm retains the structural features of the original dinosaur cells and tissue. This, combined with a biofilm's natural elasticity, gives the appearance of being soft, stretchable tissue containing cellular structures. Kaye et al. (2008) suggest that "the lack of observed cell structure in the transparent tubes is inconsistent with preserved tissues." Therefore, they conclude that such biofilms have been mistakenly identified as original dinosaur tissue that has survived millions of years without fossilizing. They further suggest that the red blood cells reported in previous studies were actually oxidized forms of pyritic frameworks.

Peterson et al. (2010) specifically address the claims of Kaye et al. (2008). They followed the development of biofilm on experimentally prepared bones, which were placed into holes in areas of the Yucatan Peninsula (Mexico). After a period of four months, the bones were removed and analyzed. The results of this study support previous suggestions that microbial activity is an important stage of fossil preservation (Briggs et al., 2003; Hollcher et al., 2001; Raff et al., 2008). Biofilms apparently metabolize organics, reducing the pH of the matrix. As a result of the lower pH, the bacteria produce minerals that form a mineral barrier (microbial masonry), which helps seal pores in the bone. This sealing may not only help accelerate the fossilization process, but also provide some protection of the inner bone tissue. In fact, microbial biofilms may actually help stabilize and preserve original tissue (Iniesto et al., 2013; Raff et al., 2014). While there is little evidence that this preservation would be sufficient for 65 million years, it may serve as at least a partial explanation for the preservation of the tissue for 4,000–5,000 years.

They further report that "fractured bones have a less-likely chance of preserving primary soft-tissues" (Peterson et al., 2010, p. 11). The researchers propose that fractured bones allow greater penetration of bacteria that can degrade any biological material. In contrast, intact bone provides only limited access of bacteria through the pores in the bone and actually enhances biofilm production of EPS and hydroxyapatite. This polysaccharide and mineral fabric seals the pores, helping protect the tissue from degradative processes.

Other studies also offer similar biofilm activity as part of the fossilization process (Briggs, 2003; Dunn et al., 1997; Daniel and Chin, 2010; Hu et al., 2011; Iniesto et al., 2013). In addition, Raff et al. (2014) propose that the type of bacteria comprising the biofilm has a significant effect on preservation quality. Hence, some tissue may be preserved and other tissue not preserved, strictly as a consequence of the bacterial population present at the time of fossilization. Hu et al. (2011) even suggest this microbial masonry event enabled body-part preservation of fossils with assigned ages of over 250 million years.

Interestingly, the study by Peterson et al. (2010) failed to provide any support for the proposal of Kaye et al. (2008). Rather, they observed a general lack of morphological similarity between samples from soft-tissue controls compared to biofilm structures (Peterson et al., 2010). Following the four months of burial, the biofilms they observed did not present any detailed vessel or cellular structures (Peterson et al., 2010), both of which have been repeatedly detected in soft tissue from dinosaur fossils (Armitage, 2015; Armitage and Anderson, 2013; Schweitzer et al., 2007, 2013). In fact, close scrutiny of microscopic biofilm images obtained by Kaye et al. (2008) reveals no intricate cellular structures with filopodia or other features that closely mimic previously reported dinosaur cells. Kaye et al. (2008) offer little explanation for the cellular detail reported by other studies, a glaring omission in their conclusions.

In addition, Peterson et al. (2010) were able to microscopically observe specific bacterial cells and EPS structures for the biofilms on their test fossils. The presence of bacterial cells in an EPS layer has never been observed in any reported examinations of pliable tissue extracted from dinosaur fossils. Rather, osteocytes, which are much larger cells than bacteria, are readily observed even without electron microscopy (Armitage and Anderson, 2013; Schweitzer et al., 2013). Thus, results from Kaye et al. (2008) are not consistent with those from other soft-tissue studies.

It should be noted that bacteria are ubiquitous on any geologic specimen. A wide variety of bacteria can participate in biofilm formation, but the simple presence of bacteria does not automatically indicate the presence of significant levels of biofilms. Under specific conditions, many bacteria appear to prefer colonizing within a biofilm community, but large and mature biofilms will not always occur. While bacteria are certainly present on the extracted pliable tissue, no distinct mature biofilms were evident in microscopic examination of various dinosaur soft/pliable tissue samples (Armitage and Anderson, 2013; Schweitzer et al. 2009, 2013). Also, as mentioned above, few biofilms become large enough to mimic the size and textural features of the extracted soft tissue.

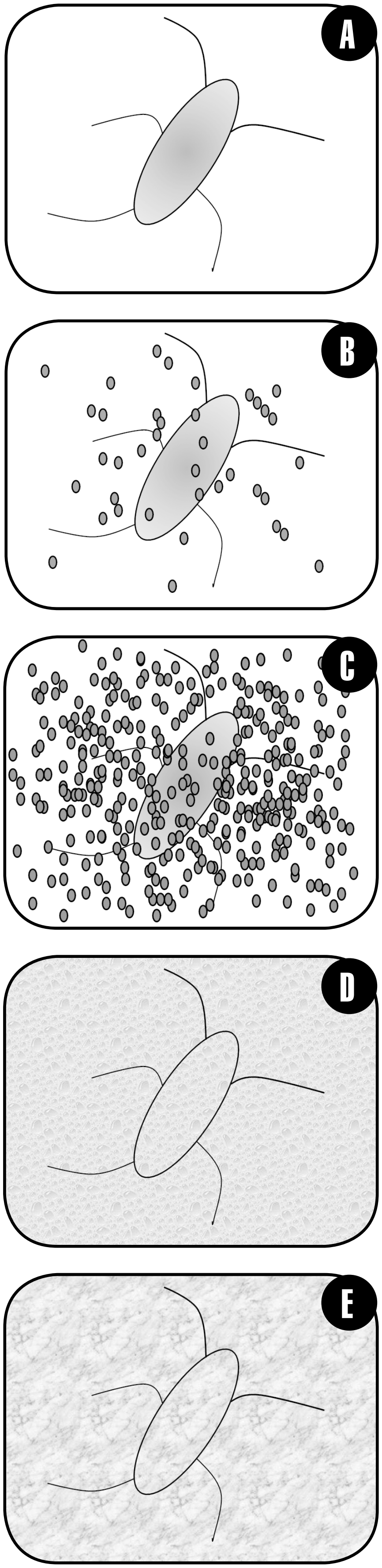

Figure 4 (*right*). Pseudomorphing model proposed by Raff et al. (2008, 2013). (A) An intact osteocyte within bone tissue. (B) During fossilization, bacteria invade bone tissue and begin to replicate their cells. (C) As the bacterial population grows, it begins releasing EPS. (D) Sufficient EPS production leads to formation of a biofilm layer over the bone tissue, which captures the impression of the osteocyte before the cell decomposes. (E) The mature biofilm is subsequently fossilized, retaining the osteocyte impression.

Kaye's model (Kaye, et al., 2008) requires that the biofilm EPS matrix can mimic characteristics of dinosaur tissue (including intact osteocytes). However, the model is vague regarding whether this structural impression occurred prior to the fossilization of the osteocytes or if the impression is that of an already fossilized cell. Raff et al. (2008, 2013) suggest that when bacteria digest tissue, they can also form a biofilm imprint of that tissue. If Kaye's model is suggesting the tissue/cell impression is made prior to fossilization, this requires the biofilm to retain extraordinary morphological detail for millions of years. It should be noted that the results of recent studies reported by Raff et al. (2008, 2013, 2014) deal with fresh specimens, and any replica or imprint they detected in the biofilm was of recent formation.

It also should be noted that the model offered by Raff and coworkers proposes what they call "pseudomorphing" (Raff et al., 2013, 2014). This involves a bacterial biofilm stabilizing the soft body parts of a dead organism and then forming an endocast of the tissue.

Subsequent mineralization preserves the biofilm as a fossilized impression of the tissue (Figure 4). They even suggest that the three-dimensional biofilm could retain some of the detailed features of the cells within the tissue (e.g., organelles, cell boundaries, etc), which allows these features to eventually be preserved in a fossilized form. However, they admit that any internal cellular detail would likely be "voids within the cells" and thus pseudomorphing may only offer a "potential" for preserving detail (Raff et al., 2013, p. 255). Regardless, this "pseudomorphing" model addresses potential mineralization leading to fossilization of soft-body parts. It does not adequately address a mechanism for preserving them as soft/pliable tissue. As such, it does not provide an adequate mechanism for Kaye's model.

Thus, biofilms may be able to retain some temporary general impressions of a surface, but there is no evidence that this impression could possess intricate cellular detail. What is more, there is certainly no evidence that a biofilm can retain an impression of detailed structures for millions of years. In fact, such a concept fully contradicts known biofilm behavior, where the biofilm structure constantly changes as a consequence of maturity, temperature, species composition, and other environmental factors. None of these factors could ever be suggested to have remained constant over a multimillion-year period. Also, Peterson et al. (2010) found no microscopic indication that biofilms could be mistakenly identified as tissue or complex cellular structures, such as osteocytes.

Close scrutiny of micrographs reported by Raff et al. (2008, 2013) reveal that bacteria and bacterial aggregates can be clearly identified within the preserving biofilm. Reports by Schweitzer et al. (2007, 2013) and Armitage and Anderson (2013) do not reveal any clearly seen bacteria or bacterial aggregates in the extracted tissue. In fact, *Triceratops* horn tissue at 800x magnification (Armitage and Anderson, 2013; figure 8–10) compared to micrographs of similar magnification reported by Raff et al. (2008, 2013) or Kaye et al. (2008) reveals very distinct differences. The pliable horn tissue possesses several cellular structures of distinct osteocyte-type cells. This is not observed in any of the biofilm images reported by Raff et al. (2008, 2013) or Kaye et al. (2008).

At even greater magnifications, the results reported by Kaye et al. (2008) and Raff et al. (2008, 2013) reveal no morphologically intricate cells in the biofilm replica. None of these studies offer electron micrographs with the level of cellular detail reported for dinosaur soft tissue (e.g., Armitage, 2015; Armitage and Anderson, 2013; and Schweitzer et al., 2013). This includes fine cellular detail to a 500-nm scale, extensive filipodia (20 µg in length), and even evidence of internal contents. The absence of such cellular detail strongly indicates a significant difference between what Kaye et al. (2008) and Raff et al. (2008, 2013) observed and what has been observed in the pliable tissue extracted from dinosaur fossils.

Bacterial Proteins?

Collagen

Kaye et al. (2008) further challenge that findings of dinosaur collagen detected in fossil samples (Asara et al., 2007) are not original proteins. Instead, they document that bacteria make a collagen-like protein (Rasmussen et al., 2003; Yu et al., 2014). Kaye's model proposes that this bacterial protein is the true origin of the collagen detected in dinosaur specimens.

To further support this position, Kaye et al. (2008) obtained C-14 dates of the pliable tissue from dinosaur specimens. Their C-14 results indicate that carbon in the biomolecules of the tissue (mostly collagen) is of recent origin. They suggest this young age is more consistent with recent bacterial origin of biomolecules in the fossil, since evolutionary interpretation would require any dinosaur tissue to be "carbon dead" (i.e., greater than one million years of age; for a more detailed discussion of the significance of C-14 in dinosaur fossils, see Thomas and Nelson, 2015).

Collagen is the most abundant protein in the animal kingdom. It is a heterogeneous family of glycoproteins that contain at least one triple helical domain. All animal collagens are composed of three alpha-chains consisting of a triple helix motif. This motif consists of a repeating sequence of three amino acids, specifically glycine followed by two other amino acids. The motif is commonly designated as (Gly-Xaa-Yaa)$_n$ (Exposito et al., 2010).

Many bacteria appear to make a collagen-like protein that also possesses a triple helical motif (Rasmussen et al., 2003). This protein can have a similar structural matrix as animal collagen. Thus, it can potentially be misinterpreted by microscopic analysis as animal collagen.

However, all known vertebrate collagen possess hydroxyproline (a modification of proline), and it is frequently found in the third position (the Yaa position) of the triple helix motif (Shoulders and Raines, 2009). Bacteria often place proline in the second position (Xaa) (Yu et al., 2014), and threonine and glutamine are frequently in the Yaa position of the helix (Rasmussen et al., 2003). In addition, bacteria lack the ability to convert proline to hydroxyproline and therefore lack this modified form of proline in their collagen-like proteins (Xu et al., 2010; Yu et al., 2011). The absence of the hydroxyproline appears to make bacterial collagen-like protein more susceptible to destabilization by temperature and pH changes (Mohs et al., 2007). Asara et al. (2007) report that approximately 50% of proline residues in putative dinosaur collagen is hydroxylated. This constitutes a clear distinction between bacterial collagen-like protein and the collagen detected in the dinosaur tissue. Thus, the proposal by Kaye et al. (2008) is contradicted by the chemical differences between bacterial protein and the collagen extracted from the fossils.

As additional confirmation of collagen, Lindgren et al. (2011) used antibody specificity to detect type I avian collagen. This antibody has an affinity for collagen isolated from dinosaur fossils but lacks affinity to bacterial "collagen" or negative controls. Thus, there is no evidence of bacterial collagen-like proteins in the pliable tissue samples from the fossils examined by these researchers.

Immunoassays of antibody affinity can be subject to false results as a consequence of (1) lack of antibody epitope specificity, and (2) inadequate microscope sensitivity. Lindgren et al. (2011) addressed the first concern by use of several positive and negative controls and use of an antibody with high specificity for animal collagen (esp. avian). The second concern was addressed by using confocal microscopy, enabling a very precise detection of antibody binding.

As further support of the detection of animal collagen, they employed synchrotron, radiation-based infrared microspectroscopy (Lindgren et al., 2011), which can analyze specific microstructures of biomolecules. This microspectroscopy found that the amino acids were located in the bone matrix fibrils. This is consistent with typical animal collagen characteristics but differs significantly from bacterial collagen-like proteins (Lindgren et al., 2011).

In addition, Schweitzer et al. (2009) also used antibodies with a specific reactivity to type I avian collagen. They found these antibodies attach to segments of the isolated dinosaur collagen samples but not to control samples. They also did a BLAST analysis of the amino acid sequences of the short collagen fragments they obtained from the fossil. While several of the sequences had some alignment with chicken, alligator, or *T. rex* proteins, none aligned with any bacterial protein sequences. Plus, Bern et al. (2009) used mass spectroscopy to reanalyze samples used by Asara et al. (2007), confirming the presence of "bird-like" hemoglobin and collagen with no evidence of contamination found in the samples. Thus, in each of these studies, there is no evidence that the detected collagen was of bacterial origin.

Actin and Tubulin

Other proteins have also been detected in dinosaur specimens. Schweitzer at al. (2013) detected proteins such as actin and tubulin. In eukaryotic cells, actin and tubulin serve as cytoskeletal proteins. Bacteria are also known to possess a cytoskeleton partly comprised of MreB and ParM—proteins that have a three-dimensional structure similar to that of actin but only a weak amino-acid-sequence similarity (Carballido-López, 2006). Bacteria also produce a protein (FtsZ) that has some tubulin-like functions and properties, but again, the amino-acid similarity with eukaryotic tubulin is very low (van den Ent et al., 2001).

There is no indication that any of these bacterial proteins (MreB, ParM, and FtsZ) would react with the immunoassay used by Schweitzer et al. (2013). In fact, these researchers analyzed both bacterial and surrounding soil samples and detected no proteins with the same antibody affinity as the actin and tubulin extracted from the pliable tissue. Thus, there is no evidence that the researchers misidentified bacterial proteins as animal actin or tubulin. Bacterial biofilms fail to offer an appropriate explanation for the presence of these proteins. This further weakens challenges that the biomolecules are bacterial contamination rather than original dinosaur proteins.

Original Tissue

As increased care has been taken to eliminate possible contamination and to account for non-dinosaur biomolecules, the results of recent studies have consistently shown (1) the presence of pliable tissue containing intact bone cells with detailed structures, (2) the presence of biomolecules, such as collagen, that appear to be original to the fossil, (3) a complete absence of evidence for bacterial biofilms mimicking tissue or dinosaur collagen, and (4) the lack of significant evidence that either the pliable tissue or the biomolecules it contains are from a contaminating source.

As a body of work, the evidence for unfossilized tissue in dinosaur fossils is significant. Attempts to suggest the tissue is contamination, biofilm, or an error of analysis can no longer be viewed as viable. That this pliable material is original dinosaur tissue sets in motion the need for explanations of its remarkable preservation. Several models have been proposed, all with the clear objective of explaining how otherwise fairly labile biological material could withstand degradation processes for multimillions of years. So far, all explanations offered fail to provide an adequate explanation (see DeMassa and Boudreaux, 2015, and Thomas, 2015 for more extensive discussion). This leaves the alternative—that dinosaur fossils are far younger than millions of years of age—as the most viable and consistent interpretation.

Acknowledgments

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References

CRSQ: Creation Research Society Quarterly

Allentoft, M.E., M. Collins, D. Harker, J. Haile, C.L, Oskam, M.L. Hale, P.F. Campos, J.A. Samaniego, M.T.P. Gilbert, E. Willerslev, G. Zhang, R.P. Scofield, R.N. Holdaway, and M. Brunce. 2012. The half-life of DNA in bone: measuring decay kinetics in 158 dated fossils.

Proceedings of the Royal Society B 279:4724–4733.

Anderson, K.L. 2003. The complex world of gastrointestinal bacteria. *Canadian Journal of Animal Science* 83:409–427.

Armitage, M. 2015. Soft bone material from a brow horn of a *Triceratops horridus* from Hell Creek Formation, MT. *Creation Research Society Quarterly* 51:248–258.

Armitage, M., and K.L. Anderson. 2013. Soft tissue of fibrillar bone from a fossil of the supraorbital horn of the dinosaur *Triceratops horridus. Acta Histochemica* 115:603–608.

Asara, J.M., M.H. Schweitzer, L.M. Freimark, M. Phillips, and L.C. Cantley. 2007. Protein sequences from mastodon and *Tyrannosaurus rex* revealed by mass spectrometry. *Science* 316:280–285.

Bada, J.L., X.S. Wang, and H. Hamilton, H. 1999. Preservation of key biomolecules in the fossil record: current knowledge and future challenges. *Philosophical Transactions of the Royal Society B* 354:77–87.

Bern, M., B.S. Phinney, and D. Goldberg. 2009. Reanalysis of *Tyrannosaurus rex* mass spectra. *Journal of Proteome Research* 8:4328–4332.

Bridier, A., F. Dubois-Brissonnet, A. Boubetra, V. Thomas, and R. Briandet. 2010. The biofilm architecture of sixty opportunistic pathogens deciphered using a high throughout CLSM method. *Journal of Microbiology Methods* 82:64–70.

Briggs, D.E.G. 2003. The role of decay and mineralization in the preservation of soft-bodies fossils. *Annual Review of Earth and Planetary Science* 31:275–301.

Buckley, M., A. Walker, S.Y.W. Ho, Y.Yang, C. Smith, P. Ashton, J.T. Oates, E. Cappellini, H. Koon, K. Penkman, B. Elsworth, D. Ashford, C. Solazzo, P. Andrews, J. Strahler, B. Shapiro, P. Ostrom, H. Gandhi, W. Miller, B. Raney, M.I. Zylber, M.T.P. Gilbert, R.V. Prigodich, M. Ryan, K.F. Rijsdijk, A. Janoo, and M.J. Collins. 2008. Comment on "protein sequences from mastodon and *Tyrannosaurus rex*. revealed by Mass Spectrometry." *Science* 319:33.

Cambell, H. 2008. Soft dinosaur tissue dispute—probably just biofilm, says study. Science 2.0. http://www.science20.com/news_releases/soft_dinosaur_tissue_dispute_probably_just_biofilm_says_study (accessed January 5, 2015).

Carballido-López, R. 2006. The bacterial Actin-like cytoskeleton. *Microbiology and Molecular Biology Reviews* 70:888–909.

Cooke, A.J., R.K. Rowe, B.E. Rittman, and I.R. Fleming. 1999. Modeling biochemically driven mineral precipitation in anaerobe biofilms. *Water Science and Technology* 39(7): 57–64.

Cooke, A.J., R.K. Rowe, B.E. Rittman, J. VanGulck, and S. Millward. 2001. Biofilm growth and mineral precipitation in synthetic leachate columns. *Journal of Geotechnical and Geoenvironmental Engineering* 127:849–856.

Daniel, J.C., and K. Chin. 2010. The role of bacterially mediated precipitation in the premineralization of bone. *PALAIOS* 25:507–516.

Demassa, J.M., and E. Boudreaux. 2015. Dinosaur peptides: preservation and degradation. *Creation Research Society Quarterly* 51:268–285.

Dunn, K.A., R.J.C. McLean, G.R. Upchurch, and R.L. Folk. 1997. Enhancement of leaf fossilization potential by bacterial biofilms. *Geology* 25:1119–1122.

Elias, S., and E. Banin. 2012. Multi-species biofilms: living with friendly neighbors. *FEMS Microbiology Reviews* 36:990–1004.

Exposito, J.-Y., U. Valcourt, C. Cluzel, and C. Lethias. 2010. The fibrillar collagen family. *International Journal of Molecular Science* 11:407–426.

Flemming, H.-C., and J. Wingender. 2010. The biofilm matrix. *Nature Reviews. Microbiology* 8:623–633.

Hecht, J. 2008. T. rex 'tissue' may just be bacterial scum. *New Scientist.* http://www.newscientist.com/article/dn14427-t-rex-tissue-may-just-be-bacterial-scum.html#.VM0bMC7-uSo (accessed January 5, 2015).

Hochbaum, A.I., and J. Aizenberg. 2010. Bacteria pattern spontaneously on periodic nanostructure arrays. *Nano Letters* 10:3717–3721.

Hollocher, T.C., K. Chin, K.T. Hollocher, and M.A. Kruge. 2001. Bacterial residues in coprolite of herbivorous dinosaurs: a role of bacteria in mineralization of feces. *PALAIOS* 16:547–565.

Hu, S. Q. Zhang, Z-Q., Chen, C. Zhou, T. Lü, T. Xie, W. Wen, J. Huang, and M.J. Benton. 2011. The luoping biota: exceptional preservation, and mass evidence on the Triassic recovery from end-Permian mass extinction. *Proceedings of the Royal Society B* 278:2274–2282.

Iniesto, M., A.I. Lopez-Archilla, M. Fregenal-Martínez, A.D. Buscalioni, and M.C. Guerrero. 2013. Involvement of microbial mats in delayed decay: an experimental essay on fish preservation. *PALAIOS* 28:56–66.

Kaye, T.G., G. Gaugler, and Z. Sawowicz. 2008. Dinosaurian soft tissues interpreted as bacterial biofilms. *PLoS One* 3(7): e2808.

Lindgren, J., P. Uvdal, A. Engdahl, A.H. Lee, C. Alwmark, K.-E. Bergquist, E. Nilsson, P. Ekström, M. Rasmussen, D.A. Douglas, M.J. Polcyn, and L.L. Jacobs. 2011. Microspectroscopic evidence of Cretaceous bone proteins. *PLoS One* 6(4): e19445.

McNamara, M.E., P.J. Orr, S.L. Kearns, L. Alcalá, P. Anadon, and E.P. Molla. 2009. Soft-tissue preservation in Miocene frogs from Libros, Spain: insights into the genesis of decay microenvironments. *PALAIOS* 24:104–117.

Mohs, A., T. Silva, T. Yoshida, R. Amin, S. Lukomski, M. Inouye, and B. Brodsky. 2007. Mechanisms of stabilization of a bacterial collagen triple helix in the absence of hydroxyproline. *Journal of Biological Chemistry* 282:29757–29765.

Morris, N.S., D.J. Stickler, and R.C.J. McLean. 1999. The development of bacterial biofilms on indwelling urethral catheters. *World Journal of Urology* 17(6): 345–350.

Peterson, J.E., M.E. Lenczewski, and R.P. Scherer. 2010. Influence of microbial biofilms on the preservation of primary tissue in fossil and extant Archosaurs. *PLoS One* 5(10): e13334.

Raff, E.C., K.L.Schollaert, D.E. Nelson, P.C.J. Donoghue, C.-W. Thomas, F.R. Turner, B.D. Stein, X. Dong, S. Bengtson, T. Huldtgren, M. Stampanoni, Y. Chongyu, and R.A. Raff. 2008. Embryo fossilization is a biological process mediated by microbial biofilms. *Proceedings of the National Academy of Sciences, USA* 105:19360–19365.

Raff, E.C., M.E. Andrews, F.R. Turner, E. Toh, D.E. Nelson, and R.A. Raff. 2013. Contingent interactions among biofilm-forming bacteria determine preservation or decay in the first steps toward fossilization of marine embryos. *Evolution & Development* 15:243–256.

Raff, R.A., M.E. Anderws, R.L. Pearson, F.R. Turner, S.T. Saur, D.C. Thomas, J.L. Eagan, and E.C. Raff. 2014. Microbial ecology and biofilms in the taphonomy of soft tissues. *PALAIOS* 29:560–569.

Rasmussen, M., M. Jacobsson, and L. Björek. 2003. Genome-based identification and analysis of collagen-related structural motifs in bacterial and viral proteins. *Journal of Biological Chemistry* 278:32313–32316.

Rendueles, O., and J.-M. Ghigo. 2012. Multi-species biofilms: how to avoid unfriendly neighbors. *FEMS Microbiology Reviews* 36:972–989.

Schuster, J.J., and G.H. Markx. 2014. Biofilm architecture. *Advances in Biochemical Engineering and Biotechnology* 146:77–96.

Schweitzer, M.H., J.L. Wittmeyer, and J.R. Horner. 2007. Soft tissue and cellular preservation in vertebrate skeletal elements from the Cretaceous to the present. *Proceedings of the Royal Society B* 274:183–197.

Schweitzer, M.H., W. Zheng, C.L. Organ, R. Avci, Z. Suo, L.M. Freimark, V.S. Lebleu, M.B. Duncan, M.G. Vander Heiden, J.M. Neveu, W.S. Lane, J.S. Cottrell, J.R. Horner, L.C. Cantley, R. Kalluri, and J.M. Asara. 2009. Biomolecular characterization and protein sequences of the campanian hadrosaur *B. Canadensis*. *Science* 324:626–631.

Schweitzer, M.H., W. Zheng, T.P. Cleland, and M. Bern. 2013. Molecular analyses of dinosaur osteocytes support the presence of endogenous molecules. *Bone* 52:414–423.

Shoulders, M.D., and R.T. Raines. 2009. Collagen structure and stability. *Annual Review of Biochemistry* 78:929–958.

Smith, T.C. 2008. Dinosaur soft tissue—just bacterial biofilm? *Aetiology Science Blogs*. http://scienceblogs.com/aetiology/2008/07/30/dinosaur-soft-tissuejust-bacte (accessed January 5, 2015).

Stickler, D., L. Ganderton, J. King, J. Nettleton, and C. Winters. 1993. *Proteus mirabilis* biofilms and the encrustration of urethral catheters. *Urology Research* 21:407–411.

Thomas, B. 2015. Original biomaterials in fossils. *Creation Research Society Quarterly* 51:234–247.

Thomas, B., and V. Nelson. 2015. Radiocarbon in dinosaurs and other fossils. *Creation Research Society Quarterly* 51:299–311.

van den Ent, F., L. Amos, and J. Löwe. 2001. Bacterial ancestry of actin and tubulin. *Current Opinion in Microbiology* 4:634–638.

VanGulck, J.F., R.K. Rowe, B.E. Rittman, and A.J. Cooke. 2003. Predicting biogeochemical calcium precipitation in landfill leachate collection systems. *Biodegeneration* 14:331–346.

Xu, C., Z. Yu, M. Inouye, B. Brodsky, and O. Mirochnitchenko. 2010. Expanding the family of collagen proteins: recombinant bacterial collagens of varying composition form triple-helices of similar stability. *Biomacromolecules* 11:348 (doi:10.1021/bm900894b).

Yu, Z., B. Brodsky, and M. Inouye. 2011. Dissecting a bacterial collagen domain from *Streptococcus pyogenes*: sequence and length-dependent variations in triple helix stability and folding. *Journal of Biological Chemistry* 286:18960–18968.

Yu, Z., B. An, J.A.M. Ramshaw, and B. Brodsky. 2014. Bacterial collagen-like proteins that form triple-helical structures. *Journal of Structural Biology* 186:451–461.

Addendum

Butler et al. (2015) have recently added to the pseudomorphoring model presented above. They note that the best preservation of soft-body parts is frequently abdominal tissue. Hence, they speculate this preservation may be a consequence of intestinal bacterial activity. Studying the decay of shrimp, the authors conclude that under specific conditions the intestinal bacteria can produce biofilms that form molds of abdominal tissue. These biofilm molds are then gradually fossilized, enabling detailed features of the shrimp's soft interior body parts (which are normally degraded quickly) to be preserved in a fossilized state. However, as mentioned above, this pseudomorphoring mechanism does not account for the prolonged preservation of tissue in a pliable, unfossilized form. Therefore, this model does not address the pliable tissue described by the iDINO project.

References

Bulter, A.D., J.A. Cunningham, G.E. Budd, and P.C.J. Donoghue. 2015. Experimental taphonomy of Artemia reveals the role of endogenous microbes in mediating decay and fossilization. *Proceedings of the Royal Society* B 282:20150476 (doi: 10.1098/rspb.2015.0476).

Creation Research Society Quarterly 2015. 51:268–285.

Dinosaur Peptide Preservation and Degradation

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Abstract

Soft tissue recovered from purportedly 68 million years old fossils of *Tyrannosaurus rex Brachylophosauraus canadensis* have been analyzed, and preservation mechanisms have been suggested by workers. A "preservation motif" based upon structure-function relationships at the molecular level is thought to explain the selective survival of soft tissue and particular peptide sequences. Preservation motifs include molecularly sheltered environments such as collagen fibril domains that offer tight molecular packing thereby conferring resistance to degradation. Sequences enriched with hydrophobic amino acid residues and depleted in acidic residues suggest another preservation pattern. More recent work by the same group adds that Fenton type reactions, arising from trace iron discovered with the bone tissue, is responsible for "fixing" the collagen for deep time survival. The present paper reviews these preservation motifs and supportive data in light of fast-degrading amino acids such as asparagine and glutamine, and oxidatively sensitive markers tyrosine, methionine, and histidine that survived the long burial age.

Introduction

Discoveries of biomolecular remnants found in ancient organisms are mounting, including the startling discovery of dinosaurian soft tissue, which has opened new lines of investigation thought impossible a few years ago. In 2005 Mary Schweitzer et al. (2005a, 2005b) reported soft tissue found in a *Tyrannosaurus rex* fossilized femur dated at 67 million years old. A detailed documentation of the discovery of soft tissue can be found elsewhere in this issue, and details of Dr. Schweitzer's story, which has been called the greatest discovery of the twenty-first century, is available (e.g., see Fields, 2006), but a brief account will be offered here.

Remarkably the recovered tissue specimen from the femur was found to be "flexible and resilient and when stretched returns to its original shape" (Asociated Press, 2012). Subsequent polypeptide analysis of the tissue showed well-preserved amino acid sequences. In late 2012, Schweitzer reported an even more startling discovery.

> Schweitzer and her team also tested for the presence of DNA within the cellular structures, using an antibody that only binds to the "backbone" of DNA. The antibody reacted to small amounts of material within

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> the "cells" of both the *T. rex* and the *B. canadensis*. To rule out the presence of microbes, they used an antibody that binds histone proteins, which bind tightly to the DNA of everything except microbes, and got another positive result. They then ran two other histochemical stains that fluoresce when they attach to DNA molecules. Those tests were also positive. These data strongly suggest that the DNA is original, but without sequence data, it is impossible to confirm that the DNA is dinosaurian. (Peake, 2012)

While the discovery is surprising, if not shocking, it may turn out to be common. Trace organic matter has been found in Mesozoic fossil bone for decades dating to the 1960s (Schweitzer et al., 2013a). In addition to discernable cells and vessels, chemical matter such as collagen fibrils, lipids and polysaccharides have been reported. Roman Pawlicki has authored or coauthored multiple papers almost 40 years on the subject of Dinosaur soft tissue studies (e.g., Pawlicki et al., 1966; Pawlicki, 1977, Pawlicki and Nowogrodzka-Zagórska, 1998). In the past decade, paleontologists have discovered proteinacious matter in a variety of fossilized remains and are using detailed chemical analysis to elucidate chemical structures.

For example, Scheweitzer et al. (2005b, p. 780) reported that organic residue was recovered from a sauropod dinosaur eggshell, noting, "We have shown that in these well-preserved fossils, the molecular structure of endogenous antigens may be preserved for more than 70 Myr." Schweitzer et al. (2007, p. 188) continued their work and reported finding "thin, flexible and fibrous, and emerged as sheets of material" in demineralized *Triceratops horridus* (MOR 699, 65 Ma) bone. (Ma and Myr mean million years ago and MOR is Museum of the Rockies).

Lindgren et al. (2011) "demonstrate that endogenous proteinaceous molecules are retained in a humerus from a Late Cretaceous mosasaur (an extinct giant marine lizard)." Cody et al. (2011) reported a signature of a "relict chitin-protein complex preserved in a Pennsylvanian (310 Ma) scorpion cuticle and a Silurian (417 Ma) eurypterid cuticle via analysis with carbon, nitrogen, and oxygen X-ray absorption near edge structure (XANES) spectromicroscopy." In addition, an international group of scientists (2012) has managed to identify 126 distinct protein sequences from a 43,000-year-old bone from a woolly mammoth (*Mammuthus primigenius*) (Cappellini, et al., 2012).

Edwards et al. (2011, p. 3217) studied a 50-million-year-old reptile concluding, "taken together, all the analyses performed in this study strongly suggest that the fossilized reptile skin… is not a simple impression, mineralized replacement or an amorphous organic carbon film, but contains a partial remnant of the living organism's original chemistry, in this case derived from proteinaceous skin."

Paleontologist Robert Reisz with the University of Toronto, Mississauga, led an international team of scientists from Canada, Taiwan, the People's Republic of China, Australia, and Germany to excavate a site in South Africa yielding a 190-million-year-old dinosaur nesting site of the prosauropod dinosaur Massospondylus. The clutch of eggs produced over 200 bones from individuals at different stages of embryonic development (Reisz, et al., 2012; 2013). Robert Reisz, the principle investigating of the work observed that "the bones of ancient animals are transformed to rock during the fossilization process … to find remnants of proteins in the embryos is really remarkable, particularly since these specimens are over 100 million years older than other fossils containing similar organic material" (Crucefix, 2013). In fact, we are not aware of any significant technical challenges to the protein identification that has been given. Also, Glass et al. (2012) identified eumelanin, an organic biopolymer that makes up the black ink found in modern squids, still present in an ink sac from a 160-million-year-old squidlike giant.

Recently, Armitage and Anderson (2013) were the first to report detection of flexible tissue and observed osteocytes in the supraorbital horn of *Triceratops horridus*. This significantly broadens the scope of specimens found to contain nonfossilized material. In fact, it would seem that numerous additional findings are forthcoming since investigators are now aware of the probable existence of organic matter in alleged ancient specimens.

Before the "Brex" Findings

Schweitzer's work prior to the discovery of a *T. rex* [museum designation of MOR 1125] was helpful and possibly profoundly preparatory for future discoveries (Schweitzer et al., 1997). In 1997, she reported that a "near-complete specimen of the Late Cretaceous dinosaur T-Rex "Big Mike" [MOR 555] was collected by the Museum of the Rockies (MOR) from the Hell Creek formation (67–65 million years ago) of eastern Montana in 1990" adding that "burial was rapid enough to forestall damage by scavenging and weathering" (Schweitzer et al., 1997, pp. 6291–6292). They eventually removed "trabecular tissues" from MOR 555 after removal of surrounding sediment from the bones. The team studied isolated protein fractions using HPLC, NMR, ESR, Raman Spectroscopy and several biochemical techniques. HPLC revealed the presence of heme-containing compounds in the bone (absorption spectrum at 405–410 nm) but not the surrounding sediments or extract solvents showing no contamination. NMR showed the "presence of a paramagnetic atom, such as those seen in various metallo-proteins. The spectrum was consistent with degraded heme proteins in the met (Fe^{3+}) state." A Raman spectra of the extracts showed marker bands consistent with the "heme prosthetic group." Using ELISA (Enzyme-Linked ImmunoSorbent Assay; see http://www.immunochemistry.com/what-immunoassay), the group reports that immunological studies reveal "striking evidence for the presence of hemoglobin derived peptides in the (*T-rex*) bone extract" (Schweitzer et al., 1997, p. 6295). "Rats immunized with *T. rex* tissue extracts produced antibodies that recognized hemoglobin" (Schweitzer et al., 1997, p. 6294), indicating the presence of hemoglobin in the *T. rex* tissue. The same response was not observed for the surrounding sandstone matrix and plant extracts. The researchers concluded that "six independent lines of evidence point to the existence of heme-containing compounds and/or hemoglobin breakdown products in extracts of trabecular tissues of the large theropod dinosaur *Tyrannosaurus rex*" (Schweitzer et al., 1997, p. 6291).

Schweitzer began to suspect that remnants of original or endogenous protein could survive in well-preserved fossils. Yet many in the community remained unconvinced. Commenting on the state of the art understanding of protein/DNA stability, she writes, "test tube studies showed organic molecules should not survive more than a million years … (and DNA less than that)" (Schweitzer, 2010, p. 67). One reviewer, she explains, rejected out of hand the possibility of long ages of protein survival whatever data she presented (Schweitzer, 2010). She followed the work with additional studies to confirm the principle of long survival ages. In 2002, she sequenced protein from mammoth fossils using mass spectrometry and "unambiguously identified collagen, a key component of bone, tendon, skin and other tissues" (Schweitzer, 2010, p. 67).

"Brex"

In 2003 the Museum of the Rockies completed excavation of a *T.rex* specimen designated MOR 1125, nicknamed "Brex" after discoverer Bob Harmon. Helicopters were needed to retrieve the remains. One of the plaster jacketed leg bones was split to enable easier removal but many fragments resulted. Schweitzer and assistant Jennifer Wittmeyer examined the fragments using the analytical and biochemical techniques developed with her earlier work. The internal structure of the bone was examined, and to their surprise, lining the inner surface of one of the bone fragments was a fibrous and channeled bone appearing to be medullary bone, which suggested that Brex was pregnant. Medullary bone appears for a very short time when a female bird is "in lay." The rich bone deposit offers a source of calcium for eggshells and is found in birds, and by extension, egg-laying dinosaurs. Demineralization was next attempted with mild acid. A fibrous-stretchy-flexible clump of tissue was left, just as observed when the procedure is applied to fresh samples of bird bones. The experiment was repeated many times. Treatment of the denser cortical (outside or sheath) bone resulted in yet greater quantities of soft tissue but this time they observed "hollow, transparent, flexible, branching tubes … [that] looked like blood vessels" (Schweitzer, 2010, p. 69). Osteocytes that secrete collagen and other organic substances found in bone were also observed.

According to Schweitzer, the results published in 2005 (Schweitzer et al., 2005a, 2005b) but did not immediately attract controversy. The next step concerned analysis of the recovered tissue. Analytical work on the "degraded" protein was attempted. The team was eventually able to sequence the material. These results published in 2007 and 2008 were met with great controversy. Schweitzer reports that they grouped most closely with birds, followed by crocodiles—the two groups that are the closest living relatives.

The dissenting claims charged that the organic matter was "slime" or a biofilm produced by microorganisms. The biofilm theory holds that bacterial polysaccharide slime displaces exogenous soft tissue inside bone cavities. The resulting product resembles soft tissue but is a product of bacteria activity. Examining numerous ancient specimens, Kaye et al. (2008) found biofilm contamination in most specimens. Carbon dating of the film points to its relatively modern origin. In addition to similarities between the infrared spectra of modern biofilms and the subject bone tissue where both had a polysaccharide signature, the group also reported the presence of blood cell size iron-oxygen spheres. The chemical identity was an oxidized form of formerly (FeS) pyritic spherical iron particles. Taken together, they concluded that the biofilm or slime is the identity of the organic residue, not dinosaurian soft tissue. This topic is covered in more detail by Anderson (2015).

Still others believed that not enough sequences were produced for an accurate analysis of the protein (Buckley, et al, 2008; Pevzner, et al., 2008). A subsequent discovery, according to Schweitzer seemed to "calm the storm." A duckbill dinosaur (*Brachylophosaurus canadensis* [Museum of the Rockies (MOR) 2598], "Brachy") was uncovered the following summer, and great precautions were taken to shield the fragile bones and contents from air pollutants, humidity changes and other possible contaminates. The finding was a treasure house of osteocytes, intact cell nuclei, and collagen fibers. The decisive evidence showed that the reportedly 80 million year old duckbill bone "reacted with antibodies that target collagen and other proteins that bacteria does not manufacture" according to Schweitzer (2010, p. 69). After receiving confirmation from 3 different labs, further criticism regarding the biofilm hypothesis ended. A subsequent paper disclosed the peptide analysis of the proteinacious material recovered from Brex (Schweitzer et al., 2011).

Peptide	Species	Chain	Amino Acid Sequence (NH_2 ....COOH)	Position	Observed	Predicted
1	T-Rex	a-1	GA***T***GAPGIAGAPGFPGAR	220–237	0	2.1
2	T-Rex	a-1	GAAGPPGA***T***GFPGAAGR	687–704	0	2.0
3	T-Rex	a-1	GV***Q***GPPGP***Q***GPR	508–519	0	1.4
4	T-Rex	a-1	GVVGLPG***Q***R	781–789	0	1.0
5	B-Can	a-1	GL***T***GPIGPPGPAGAPG***DK***G***E***AGP***S***GPPGP***T***GAR	586–618	2	3.8
6	B-Can	a-1	G***S***AGPPGA***T***GFPGAAGR	687–704	0	2.0
7	B-Can	a-1	GA***T***GAPGIAGAPGFPGAR	220–237	0	2.1
8	B-Can	a-1	GV***Q***GPPGP***Q***GPR	508–519	0	1.4
9	B-Can	a-1	GP***S***GP***Q***GP***S***GAPGPK	238–252	0	1.7
10	B-Can	b-2	G***SN***G***E***PG***S***AGPPGPAGLR	292–309	1	2.0
11	B-Can	b-2	GLPG***ES***GAVGPAGPPG***S***R	502–519	1	2.0

Table 1. List of collagen I peptides found in *Tyrannosaurus rex* and *Brachylophosauraus canadensis* trabecular bone specimens. Reproduced from San Antonio et al. (2011). *Amino acids in bold font* are potentially reactive (authors' insert).

Soft Tissue Analysis

Bone is an organic-mineral composite comprising of mainly collagen I, hydroxyapatite—[$Ca_5(PO_4)_3OH$]—a mineral substance, and populations of cells. The cell types include osteoblasts responsible for producing bones and osteocytes that maintain and remodel bone (Schweitzer et al., 2013b). Schweitzer was well aware of conventional studies that showed fast decomposition rates for the organic phase and especially DNA. Model studies showed an upper theoretical limit of ca.100,000 years for DNA and ca.3,000,000 years for proteins at low temperatures (10°C, 50°F) (San Antonio et al., 2011). Some evidence suggested that DNA can survive thousands of years past the predicted limit (e.g. recovery of a complete, "high-quality mitochondrial [mt] genome from a stratigraphically validated 130,000- to 110,000-year-old polar bear jawbone" [Lindqvist, et al., 2010]). Schweitzer concluded that the degradation models were inadequate to explain deep time survival given the accepted view that the specimens were 68 million years old. Yet, their analysis successfully showed the dinosaurian peptide sequences drawn from *Tyrannosaurus rex* and *Brachylophosauraus canadensis*. In two studies, they reported the recovery of collagen I and osteocytes and sequencing of these protein materials (Table 1 and 2). The first report appeared in 2011 concerning the collagen I mass spectral (MS) data (Table 1) and the second in 2013 concerning MS data for protein segments found in osteocytes (Table 2).

In the second report, Schweitzer et al. (2013b) more closely examined the "soft, transparent microstructures" appeared to be osteocytes. They reported immunological and mass spectral evidence consistent with extant proteins including actin, tubulin, PHEX and histone H4. They "reanalyzed mass spectrometry data collected on tryptic digests of extracts of powdered dinosaur bone, containing osteocytes as well as other cell and tissue types, from *T. rex* and *B. canadensis* to support the presence of actin, tublin and *PHEX*… Osteocytes are characterized by expression of several proteins including…actin, tubulin…and proteins that play a role in bone mineralization, such as *PHEX*" (Scheitzer et al., 2013b, p. 418). The table presented here (Table 2) summarizes data from Schweitzer's report. We will highlight some chemically active amino acids discussed below.

It is also noteworthy that Cleland et al. (2012) published an excellent work concerning the evaluation of protocols used for their specimens. Well aware of the potential to alter the soft tissue remains, they attempted to review known analytical procedures that were considered "clean, efficient extraction (methods) to obtain high quality data" (Cleland et al., 2012, p. 1). Firstly, recovery of the bone protein requires removal of the inorganic bone portion known as hydroxyapatite [$Ca_5(PO_4)_3OH$] with which the tissue is bound. Demineralization is accomplished using dilute strong acid such as HCl or ethylene diamine tetraacetic acid (EDTA). Hydrochloric acid while removing the inorganic matrix has

Peptide	Species	Type	Amino Acid Sequence (NH$_2$ ....COOH)*	Mass
12	*B. canadensis/T. rex*	Actin	AGFAG***DD***APR	976.448
13	*B. canadensis*	Actin	AVFP***S***IVGR	945.552
14	*T. rex*	Tubulin a -1A	***Q***[-17]LF***H***P***EQ***LI***T***GK	1393.747
15	*B. canadensis/T. rex*	Tubulin a -1A	***E***II***D***LVL***D***R	1085.620
16	*B. canadensis*	Histone H4	VFL***EN***VIR	989.578
17	*B. canadensis*	Histone H4	***T***V***T***AM[+16]***D***VV***Y***ALK	1326.697
18	*B. canadensis*	Histone H4	***T***L***Y***GFGG	714.346
19	*B. canadensis/T. rex*	Histone H4	ISGLI***YEET***R	1180.621
20	*B. canadensis*	Histone H4	***D***AV***TY***T***EH***AK	1134.543

Table 2. List of actin, tubulin, and histone peptides found by mass spectrometry in dinosaur bone samples. Reproduced from Schweitzer et al. (2013b). *Amino acids in bold font* are potentially reactive.

the unfortunate side effect of degrading the protein by hydrolysis. This particular reagent for example was not used in the recovery of peptides shown in Table 2. EDTA, which was used, releases the phosphate into solution and thereby frees the protein. The next step requires extraction of the protein into solution for which guanidine hydrochloride was employed. This reagent denatures proteins into random coils increasing their water solubility. The final step is lyophilization, or freeze-drying, leaving the protein as a powder isolate (Cleland et al., 2012). For mass spectrometric work presented in the tables, a tryptic enzyme digest treatment of the powdered protein is lastly applied to cleave the polypeptide at known amino acid locations, arginine (R) or lysine (K) (Schweitzer et al., 2013b). The smaller fragments are then analyzed.

Preservation Motifs

Eleven fossil peptides in Table 1 were identified which comprise less than fifteen percent of the length of the collagen triple helix known in the human/rat database (San Antonio et al., 2011). Some trends were observed. Firstly, the general observation that proteins are intimately bound to the mineral bone matrix or biomineralized is thought to provide a significant defense against potential degradative influences (Schweitzer et al., 2011; Cleland et al., 2012). The collagen I protein-inorganic matrix complex cannot be infiltrated by large postmortem enzymes, for example, thereby reducing digestion of the protein. Secondly, the team also speculates that absorption of biomolecules on minerals may reduce degradation. They also propose that protein-bone matrix fossil sequences were from regions known to be deficient in acidic residues and enriched in hydrophobic residues, which retards hydrolysis. In addition,

> one peptide (Peptide 4) mapped to the Matrix Metalloproteinase-1 (MMP-1) cleavage domain crucial for collagen remodeling, and a site for fibronectin binding. In living tissues, the integrin binding site and MMP-1 cleavage/fibronectin binding sequences are somewhat buried under the surface of the collagen fibril. (San Antonio, et al., 2011, p. 3)

According to San Antonio, et al. (2011), the results imply that deep time preservation is enhanced by these mechanisms. Overall, they suggest that the nonrandom pattern common to both therapods suggests molecular motifs or themes important to survival of the sequences.

The osteocyte peptides (Table 2) were thought to be preserved through other protective mechanisms (Schweitzer et al., 2013b). The fragments represented originating from osteocytes suggest an absence of "necrotic or apoptotic cells which are likely destroyed rapidly by phagocytosis or by microbial attack in the post-mortem environment" (Schweitzer et al., 2013b, p. 423).

> Osteocytes [or peptide fragments] are inaccessible to other live cells, which may, in part, explain their preservation in these ancient tissues…. Osteocyte expression of apoptotic repressor proteins may also contribute to their persistence. The association of actin with alpha-actin and fibrin confers stability to actin over the lifetime of the cell and may also stabilize the protein after death. Finally, osteocytes have

> limited access to oxygen within the bone matrix, and may thus be protected from oxidative damage…. Iron, released post-mortem from hemoglobin and myoglobin through autolysis/degradation of red blood cells and muscle tissue, would act to "fix" both tissues and molecules, a hypothesis also put forth by others…. Iron is a reactive oxygen species (ROS), and this switch triggers the formation of hydroxyl radicals. Through a cascade of events referred to as Fenton chemistry, highly reactive hydroxyl radicals trigger both crosslinking of proteins and peroxidation and crosslinking of the fatty acids making up cell membranes. Because osteocytes are intimately linked through filopodia to the vascular system of bone … and because the iron-binding protein ferritin has been identified in this cell line they would be susceptible to this chain reaction. (Schweitzer et al., 2013b, p. 421).

Schweitzer's group tested this Fenton chemistry proposal in light of detecting iron associated with dinosaurian cellular material using TEM (Schweitzer et al., 2013b).

Fenton Chemistry and Tissue Preservation

The working hypothesis was simple. The iron present in cellular materials available from hemoglobin, myoglobin, cytochromes and non-heme iron proteins such as ribonucleotide reductase, fatty acid desaturase and ferritin served as causative factors in fixing proteins for long term survival. Schweitzer believed that that iron biomaterials intimately associated with surrounding soft tissue produced hydroxyl radicals which penetrate the soft tissue and cross-linked the protein thereby "fixing" the tissue. The process is similar to the fixing action of formaldehyde, which results in increasing resistance to microbial digestion, enzymatic digestion or other degradative processes (Schweitzer et al., 2013a). This study shows additional microscopic evidence that iron is closely associated with still soft tissues and their semitransparent, pliable vessels and microstructures. Additionally the group tested the hypothesis by soaking and incubating postmortem ostrich blood vessels in a red blood cell lysate rich in solubilized hemoglobin (HB). The control specimens included the same ostrich blood vessels in distilled water. Both the controls and test specimens were held under oxygenated or deoxygenated conditions. Hemoglobin was a chosen because of its "preservation properties" according to Schweitzer (Schweitzer et al., 2013a, p. 5).

Four notable properties of solubilized hemoglobin include (i) bacteriostatic properties, (ii) oxygen + HB produces free radicals, (iii) after death large vertebrates release large amounts of HB which lyse, (iv) degraded HB releases heme and iron contents to surrounding cells. Using transmission electron microscopy (TEM) and scanning electron microscopy (SEM) the group observed the condition of the cells over a two-year period. The stability of the tissue was judged by the relative appearance, which demonstrated the following: After nearly 2 years at room temperature in the presence or absence of oxygen the HB vessels showed almost "no change" and were "intact" compared with fresh specimens. The comparable controls lacking HB and soaked in water were "significantly degraded in 3 days" (Schweitzer et al., 2013a, p. 5). The control specimens were judged to have "extensive tissue degradation." Some of the signs of degradation included vessel wall thinning, bacilliform infection, fungal invasion, and vessel content reduction.

The overall judgment was that the HB-oxygen combination led to the greatest tissue stability over the two years. The deoxygenated-HB ostrich tissue specimens also appeared stable. The general conclusion was that "the hypothesis that iron contributes to preservation in deep time, perhaps by both free-radical-mediated fixation and antimicrobial activity, is supported by data presented" (Schweitzer et al., 2013a, p. 7). The group presents several possible additional routes of stabilization. The iron blocks active sites on proteins preventing enzymatic action or oxygen binding (antioxidant) or "outcompeting bacterial mechanisms" (Schweitzer et al., 2013a, p. 7). The preferred if not more discussed model involved deep time preservation through "protein and lipid cross-linking" via hydroxyl radical. They additionally speculated that other metals may contribute to hydroxyl radical production and subsequent "exceptional fossil preservation" (Schweitzer et al., 2013a, p. 8).

Analysis of Preservation Motifs

Schweitzer et al. (2013a, 2013b) have proposed numerous possible explanations for the survival of recovered collagen and other soft tissue materials found in *T. rex* and *B. canadensis* including for example molecular sheltering, hydrophobic enrichment, iron-protein blocking, cross-linking, etc. Undoubtedly all if not most of the proposals have some merit. However, in our view, the matter of these mechanisms explaining deep time survival has not been adequately supported either empirically or by literature review. We begin by merely pointing out that the Fenton chemistry hypothesis supported by the ostrich tissue preservation experiment over 2 years, using hemoglobin as a preservative is simply inadequate to extrapolate and infer stabilization over 68 million years. It is unknown if environmental factors like high-low temperature cycling or dehydration might radically alter the test specimens appearance. Is a tissue specimen soaked in blood kept in a laboratory an adequate experiment to model the environmental weathering of postmortem tissue? In this vein, it is

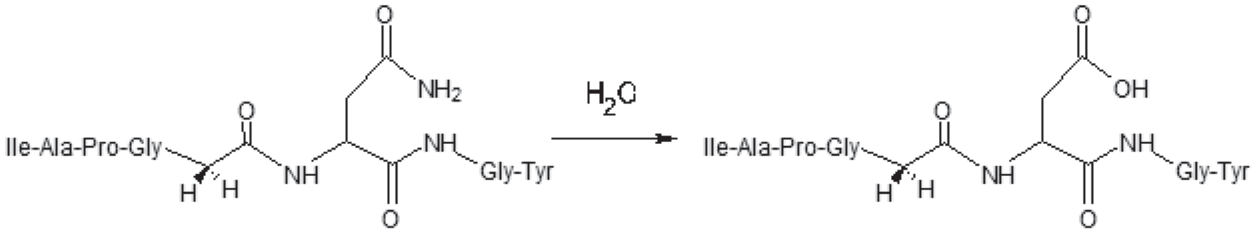


Figure 1. Deamidation of a model octapeptide.

again trivially pointed out that the visual inspection method of tissue analysis is woefully inadequate to draw any conclusions concerning a molecular mechanism of stabilization. The group has access to mass spectra evaluation which could have identified footprints of hydroxyl radical presence. As we shall see, a more careful analysis of the mass spectral data related to the particular peptides and sequences shows that some doubt, if not complete rejection, of several preservation motifs is warranted. The particular motif that introduces more problems than it purportedly solves concerns Fenton chemistry iron fixation of the peptides.

The proposal is essentially hydroxyl free-radical infiltration into soft tissue. The free radicals are generated by iron-biominerals with which the tissue is combined. Success of this mechanism depends upon deployment of the free radicals through an aqueous medium in contact with the polypeptide. We hold that "chemical fingerprints" of this activity should be registered upon the peptides themselves. For example, hydrolytically sensitive amino acids (asparagine, glutamine) should have degraded and free-radical sensitive compounds (tyrosine) should have reacted. These observations may seem like an unnecessary if not insignificant detail to observe but recall that the specimens have been in the ground for some 68 million years. If a chemical mechanism (Fenton chemistry or iron mediated hydroxyl radical fixation) is to be believed, its entire consequent (fugitive water and hydroxyl radicals dosing the peptide remnants) ought to have occurred. Below we set out upon an inspection of two general ideas concerning the presence of water and hydroxyl free radicals and their potential signature upon peptide chemistry.

Peptide Chemical Sensitivity

First, we note that Schweitzer et al. (2013a, 2013b) rightly identified molecular sheltering and hydrophobic character as likely mechanistic candidates for soft tissue survival. However, the proposal seems to run into trouble when one considers the eons of time where the specimens are thought to have laid exposed to elements such as oceanic water. The literature is particularly clear that peptide bonds on exposure to water are hydrolyzed; certain amino acids degrade very rapidly upon contact or shortly afterward with water. These studies potentially have bearing on the likelihood of proposed mechanisms to account for postmortem soft-tissue survival over 68 million years. We are not aware that Schweitzer and her coworkers have discussed the effects of these reactive amino acids, which we shall analyze below.

Asparagine and Glutamine

The reported dinosaurian peptide sequences contain asparagine (Asn, N) and glutamine (Gln, Q) which are chemically reactive amides, unlike the relatively unreactive nonpolar residues highlighted by San Antonio et al. (2011). These chemically reactive species apparently survived some 68 million years variously in the presence of Fe^{2+}, Fe^{3+}, water and hydroxyl radicals, according to Schweitzer (Schweitzer et al., 2013a, 2013b). Numerous studies show the instability of these amino acids in the presence of water. Amino acids will follow three letter shorthand abbreviations in the text and one letter symbols in the tables following below.

Asparagine: Hydrolytically Stable?

Firstly, asparagine (Asn) is hydrolytically unstable. Klotz and Thomas have studied the kinetics of deamidation of Asn for a model octapeptide (Ile-Ala-Pro-Gly-Gly-Asn-Gly-Tyr) (Klotz and Thomas, 1993). The group reports deamidation occurs under fairly mild conditions (60°C in 0.1M NaPO4, pH 7.4) to form Ile-Ala-Pro-Gly-Gly-**isoAsp**/Asp-Gly-Tyr. The group proposes that the water can attack the side chain (shown in the reaction) or the "main" chain amide group can attack the side chain (shown below). The half-life for this octapeptide was 2.17 h with an activation energy of 18.6 kcals (Payne and Manning, 2009) (Figure 1).

Robinson and Robinson (1991, p. 8882) call the sequence Asn-Gly "the most rapidly deamidation sequence known" and the triplet sequence SNG

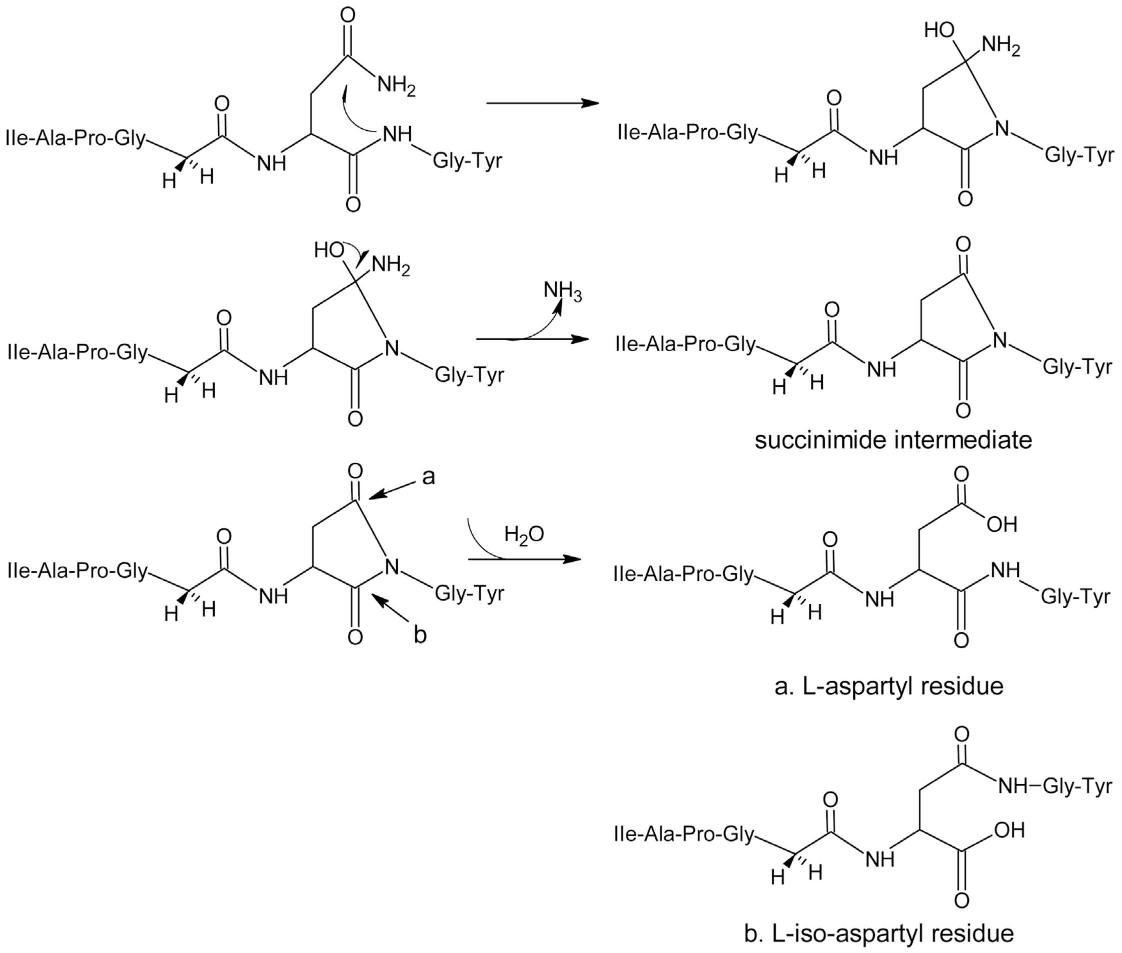


Figure 2. Deamidation of Asn-Gly through a succinimide intermediate.

among the least probable occurring, due to its instability. SNG was found in *B. canadensis* (peptide 10, Table 1).

Catak et al. (2009) suggests that the pathway of deamidation proceeds through direct hydrolysis of the Asn side chain or hydrolysis of a succinimide intermediate as shown in the diagram. Sang-aroon and Ruangpornvisuti (2013) have demonstrated in more recent work that the reaction likely involves a succinimide intermediate followed by hydrolysis (Figure 2).

Given the great hydrolytic instability of Asn, found in *B. canadensis* b-2, how did this unstable amide avoid hydrolytic or intramolecular attack for tens of millions of years? Perhaps, it might be assumed, the fragment was sheltered in some way from waters of hydration, thereby preventing hydrolysis. There undoubtedly was some sheltering, given that peptide fragments were recovered, but were these fragments also sheltered from "protective" agents for 68 million years? Schweitzer et al. (2013a, 2013b) proposes that Fenton chemistry actively cross-linked polypeptides via hydroxyl free radicals. This assumption rests upon the efficient delivery of hydroxyl species through an aqueous medium and it would seem within the peptide matrix. The dilemma is this: how did the fragment successfully become cross-linked through aqueous hydroxyl free radical attack apparently explaining peptide survival while hydrolytically unstable moieties such as Asn avoid contact with the aqueous medium—for 68 million years? If we are to accept the benefits of random aqueous hydroxyl radicals cross-linking the peptide matrix in an undefined chemical bonding, we should also accept the cost—peptide and amino acid hydrolysis.

Aspartic Acid: Out-of-place Remnant?

What can be said about the intramolecular reaction? In fact, about 25% of the reported polypeptide fragments contain L-aspartyl residue (Table 1 peptide 5; Table 2 peptides 12, 15, 17, 20), but L-*iso*-apartyl residue was not found. Assuming the presence of both products indicates the action of the intramolecular process above, we might assume the intramolecular process did not proceed, and that the L-aspartyl residue is native to the peptide sequence. This is a reasonable enough hypothesis, but the presence of the L-aspartyl residue *alone* is again problematic in light of its further potential reactions, the purported great age of the specimen, and the need for Fenton chemistry to operate.

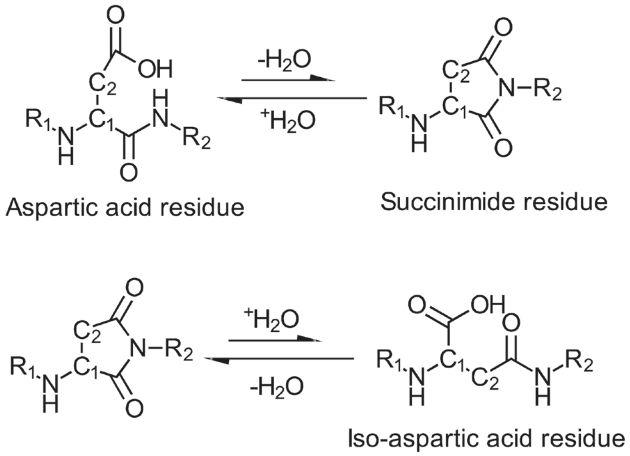


Figure 3. Isomerization of aspartic acid to isoaspartic acid via a succinimide intermediate.

Cournoyer et al. (2005) report the isomerization of aspartic acid residues to isoaspartic acid under mild conditions studying short peptide model systems (e.g. RAAAGADGDGAGADAR). Their work was done in an effort to understand protein aging. The side chain aspartic acid participates in a chain transfer event whereby the -NH-R_2- group on the C_1 "side" attacks the carboxylic acid on the C_2 "side" resulting in an iso-aspartyl residue. In Figure 3 the change can perhaps easily be seen by marking the changes as C_1 and C_2, or carbons alpha and beta, respectively, to the amide nitrogen.

The group reports, "the common, and largely accepted, model for this nonenzymatic, post-translational modification is that it spontaneously occurs under physiological conditions through a succinimide intermediate whose rate is affected by both its amino acid sequence and three-dimensional structure" (Cournoyer et al., 2005, p. 453).

What kinds of three-dimensional structural types discouraged hydrolysis for the dinosaurian proteins? Schweitzer and coworkers observe that at least one peptide (Peptide 4, Table 1) mapped to a "somewhat buried" location under the surface of the collagen fibril from which the peptide came (San Antonio et al., 2011, p. 3). They speculate that the "'sheltered' environment required to protect crucial biological function may also account for enhanced survival of those protein regions in fossils" (San Antonio et al., 2011, p. 3). They also mention biomineralization or intimate binding between the protein matter and inorganic hydroxyapatite discouraged hydrolysis or proteolytic degradation by blocking access routes to the protein. From this it can be inferred that the surviving peptides were either not accessible to water molecules or very minimally so. There is support for the sheltering model in Cournoyer et al. (2005), which teaches that hydrolytic deamidation depends upon the accessibility of water molecules to the amino acid residues without which hydrolysis proceeds at a slower rate. Cournoyer et al. (2005, p. 453) specifically cite an earlier work by Robinson and Robinson (1991) where "deamidation of an asparaginyl residue within the a-helix of rabbit muscle aldolase experienced a 15-fold slower half-life than that of its linear tetrapeptide model." The former polypeptide (aldolase) positions asparaginyl residue in a larger protein structure than the linear tetrapeptide, thus inhibiting access of water molecules to the residue. Yet it should be understood that while some support for delayed hydrolysis might be inferred from the work of Cournoyer, it is also wise to remember that a "slower half-life" deamidation reaction does not mean an arrested deamidation reaction. For Schweitzer's proposal to work, water would seemingly have to be absent.

Cournoyer et al. (2005) also teaches that less bulky side chain elements such as histidine and glycine favor hydrolysis. Two peptide sequences (Peptide 5 and 12, Tables 1 and 2 respectively) were found to contain glycine (G) where G immediately preceded aspartic (D) in both cases. Histidine (H) while present on one of the peptide sequences was at a distance that would have not have an influence on the hydrolysis mechanism (Peptide 20, Table 2). Cournoyer et al. (2005, p. 453) report that the presence of G causes a "100 fold increase in hydrolysis."

The larger point is that this particular intramolecular transformation (aspartyl residue deamidation) occurs under mild conditions without enzymatic intervention to form the iso-aspartyl acid residue. It is a neighboring group reaction facilitated by the presence of water. Again, given Schweitzer's Fenton chemistry proposal, which requires mild aqueous conditions, and according to the proposal, acting over 68 million years, is it not surprising that isoaspartic acid did not form? Note that this is an intramolecular transformation and needs only mild aqueous conditions. It would seem we need to be stronger in our requirements to allow the proposal to stand. Anhydrous conditions are required to account for the absence of isoaspartic acid—so it would seem—to shut off deamidation, but where does that leave the Fenton chemistry hypothesis? Schweitzer's model must balance molecular sheltering, Fenton chemistry, and the rigorous exclusion of water, which it does not.

Neighboring Group Destabilization

Robinson and Robinson (1991, p. 8883) report that, "in pentapeptide measurements under physiological solvent conditions, deamidation half-times ranging from 1 day to more than 9 years have been measured." Further they observe that steric hindrance plays a role whereby

> the side chain of the residue immediately preceding the amide residue was shown in the series of deamidation half-times Gly < Ala < Leu < Phe < Ile < Val with a range of 418–3278 days for Gln peptides and Gly < Ala < Val < Leu < Ile with a range of 87–507 days for Asn peptides. By contrast, a charged side chain in the residue immediately preceding the amide residues increases the rate of deamidation as was shown by the series of half-times His < Asp < Glu < Lys < Arg with a range of 96–389 days for Gln peptides and Arg < Asp < His < Glu < Lys with a range of 18–61 days for Asn peptides. Also, there are catalytic effects of neighboring hydroxyl groups from Thr (T) and Ser (S). (Robinson and Robinson, 1991, p. 8883)

Given the instability of certain triplet sequences, the group also reports that certain triplet couplings are so unstable that their frequency of occurrence becomes greatly reduced. "The five most rejected neighbors to Asn and Gln in order of the most to least rejected are Asp, Thr, Ser, Lys, and Glu" (Robinson and Robinson, 1991, p. 8882).

The general observation is that a variety of residues accelerate deamidation of Gln (Q) and Asn (N) including Thr (T), Ser (S), Val (V), Ile (I), Arg (R), His (H), Lys (K), Asp (D), and Glu (E). Inspection of the bolded amino acids in Tables 1 and 2 shows the presence of these amino adjacent to or near Gln and Asn. In Table 1, Gln is adjacent to V (peptides 3, 8), or R (peptide 4) or G (peptide 8, 9) and the SN combination is found in peptide 10. In Table 2, the triplet sequence EQL is found (peptide 14).

Regarding the unstable nature of the pairs, Robinson and Robinson (1991, p. 8883) offer the following remarks:

> Why should these unstable residues (Asn, Gln) be included in most protein molecules? Our hypothesis is that their instability is in fact their principal biological function—that deamidation serves as a molecular clock for biological processes.

Arrested Molecular Clocks?

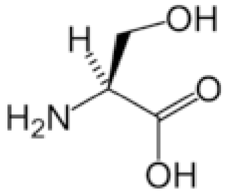
While it is reasonable to draw from Robinson that sensitive or unstable protein sequences do not survive short term physiological conditions, do these studies tutor our understanding of proteins encapsulated in buried bone fragments purportedly 68 million years old? Further are these studies helpful in light of environmental conditions consisting of some combination of heat, light, hydration, oxidation, dehydration, or microbial presence? Robinson and Robinson (1991, p. 8883, emphasis added) provide the note of caution:

> A nearest neighbor effect on deamidation can be diminished or enhanced by preferences in bond angles impressed upon the amide and its neighbors, by the availability of solvent molecules to the amide residue, by the presence of specific peptide or solvent ions, by side chain groups that sterically hinder or enhance reaction intermediates or reaction products and by association of the neighbor groups with other molecules or organelles in the system. These effects depend upon three dimensional as well as primary structures. Therefore, even if we focus our attention on the residues immediately adjacent to Gln and Asn in the peptide chains, *numerous higher-order factors can affect deamidation* rate.

Is molecular sheltering one of the "higher-order factors" that has affected deamidation for Asn and Gln? Undoubtedly the presence of these amino acids (Gln, Asn) requires some combination of complexation, structural folding and sheltering to protect them from hydrolysis whatever age is assigned to relic dinosaurian protein. Yet the studies cited above suggest that neighboring group affects and common hydrolysis are not favorable to deep time survival. The obvious question is what complexation, protein folding or sheltering effectively shut off these degradation motifs for tens of millions of years?

Serine Mediated Degradation

Another residue that destabilizes peptides in aqueous media is serine (Ser, S).



The potential problem for protein survival posed by the side group of this amino acid is demonstrated by the synthetic protein Gonadorelin. "In the pH range 5–6, the peptide backbones of gonadorelin and triptorelin are hydrolyzed at the *N*-terminal side of the serine residue probably involving nucleophilic addition of the serine hydroxyl group to the neighboring amide bond forming a cyclic intermediate resulting in *fragmentation*" (Avanti, 2012, p. 19, emphasis added). Gonadorelin is shown. The proposed cleavage apparently occurs at the carbonyl to the left of the serine residue (arrow) (Figure 4).

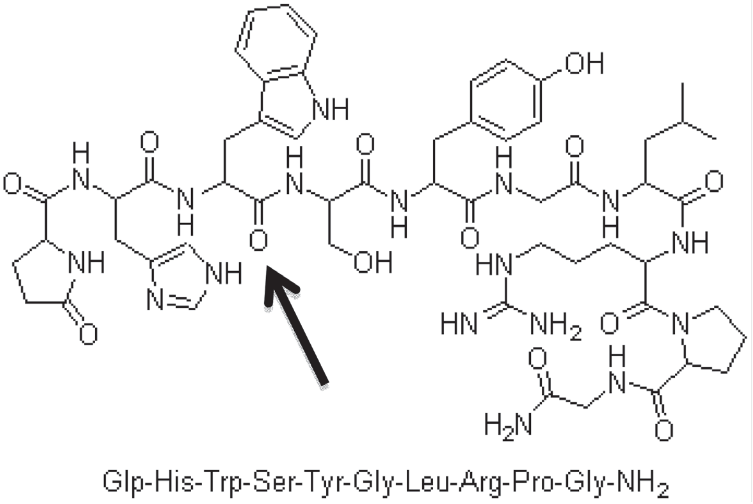


Figure 4. Gonadorelin is sensitive to hydrolysis at the carbonyl shown (arrow). The reaction is facilitated by the adjacent serine residue appearing to the left of the arrow.

Avanti's findings suggest that serine residues may destabilize peptide chain segments of great age. Five of the reported eleven peptides have serine residues (5-B-Can a-1; 6-B-Can a-1; 9- B-Can a-1; 10-B-Can a-2; 11-B-Can a-2). If the pH values in the postmortem collagen fibrils were slightly basic, it would seem serine would destabilize peptide sequences also. The stabilization mechanism proposed by San Antonio et al. (2011) presumably is that serine too was complexed or shielded sufficiently to suppress neighboring group reactions for the entire burial period. But what agent is responsible for this? For example, hydrophobic residues are not expected to stop side serine from interacting with adjacent carbonyl groups. Again it is indeed unclear how basic reactions and in this case neighboring group interactions were suspended for epochs of time.

Fenton Reaction

Finally, we turn our attention to the prospects for iron stabilization of dinosaur soft tissue, as proposed by Schweitzer et al. (2013a). A brief review of Fenton chemistry is necessary to understand Schweitzer's proposed mechanism of preservation.

In his original procedure Fenton oxidized tartaric acid by reacting it in aqueous acetic solution containing Fe^{2+} ions, plus H_2O_2, thus producing •OH (hydroxyl radicals), H_2O and Fe^{3+} ions. The •OH radicals were the active oxidizing agents in the process, which completely decompose the reactant to CO_2 and H_2O. However, excess H_2O_2 reacts to form more HO radical and HO^-, which react with the Fe^{3+} reducing it back to Fe^{2+}, $H^+_{(aq)}$ and $HOO\bullet_{(aq)}$ (peroxyl radical), another powerful oxidizing agent. If these two reactions occur simultaneously, the net result is

$$2H_2O_2\ (aq) \rightarrow HO\bullet + HOO\bullet + H_2O \tag{1}$$

Since both the hydroxyl and peroxyl radicals are available, the likelihood is that any organic substrate will be completely decomposed to its oxide products plus water.

If the initial Fe^{2+} lacks sufficient acid and /or hydrogen peroxide (one mole H^+ and H_2O_2 per mole Fe^{2+}) the oxidation to Fe^{3+} together with the HO• and H_2O will be incomplete. Consequently, the amount of HOO• and H^+ will be limited as well. Barb et al. (1951) subsequently showed that Fenton like oxidations were critically dependent on the ratios of Fe^{2+} to H_2O.

Biomolecular systems containing iron are primarily confined to the cytochromes, ferredoxin, hemoglobin and myogolbin. These are symbolically represented as L-Fe^{2+} and L-Fe^{3+}, where L is the biomolecular ligand. In such systems, the redox mechanism is believed to be initiated via a superoxide ion, $\bullet O_2^-$, which is generated in the decomposition of organic matter and functions either as an oxidant or reductant, depending on what it reacts with. According to Prousek (2007) the pertinent equations are

$$\text{L-Fe}^{3+} + \bullet O_2^- \rightarrow \text{L-Fe}^{2+} + O_2 \tag{2}$$

$$\text{L-Fe}^{2+} + H_2O_2 \rightarrow \text{L-Fe}^{3+} + HO\bullet + HO^- \tag{3}$$

$$\text{Or L-Fe}^{2+} + H_2O_2 \rightarrow \text{L-FeO}^{2+} + H_2O \tag{4}$$

However, we do not believe that equation (4) is a viable option, since Fe in the 4+ valence state is not readily stabilized. Also, in equation (4) the L-FeO^{2+} is also a very powerful oxidizing agent.

Such redox processes, as those listed above, are known to induce extensive oxidative damage to biological systems (Prousek, 2007). Furthermore, oxy radicals and the Fe^{2+} cation (equation (4) are so destructive to biological systems that Prousek (2007, pp. 2328–2329) said, "Due to its strong reactivity with biomolecules, hydroxyl radical is capable of doing more damage to biological systems than any other reactive organic species."

HO• Cross Linking Reactions

The work of Dizdaroglu and Gajewski (1989) cite a number of publications involving HO• as a cross-linking reagent in biomolecules, but these studies all relate to controlled irradiative techniques for generating the hydroxyl radicals within samples that were carefully prepared and buffered. But as we have already noted, given unregulated conditions, the destructive consequences of hydroxyl radical reactions are paramount and will certainly take precedence over any controlled cross-linking reactions.

It is interesting, that there do not appear to be any published studies in which nonliving tissue was observed to produce cross-linking within biomolecules, under simulated conditions of the natural environment. Nonetheless, Schweitzer et al. (2013, p. 2) explicitly state, "oxy radicals facilitate protein cross-linking in a manner analogous to the action of tissue fixatives (e.g. formaldehyde), thus inducing resistance of these "fixed" biomolecules to enzymatic or microbial digestion." However, the reference citation given in the Schweitzer et al. (2013a) paper is that of Hawkins and Davies (2001), which cites 240 publications all relating to bioenergetic deleterious reactions of radicals primarily on proteins. This is hardly an endorsement to the statement quoted above.

The work of Schweitzer et al. (2013a) hinges upon a natural, uncontrolled production of hydroxyl radicals involving iron containing molecules, via reactions (2) - (4) above, which will selectively promote cross-linking, thereby affording stabilization of dinosaur tissue toward degradation. But it has been shown that the bulk of all relevant evidence strongly suggests that it is highly unlikely for such cross-linking to occur. On the contrary, it is most likely that the involvement of such reactive radicals would lead to greater decomposition, rather than stabilization. Whichever the situation, neither direct evidence nor a model study have been offered to support this cross-linking claim.

Hydroxyl Radical Markers

Perhaps the most questionable aspect of the Fenton chemistry hypothesis of cross-linking or protein fixing arises from the data itself. If hydroxyl radical randomly reacted with peptides, it would seem that various amino acids should have registered the change. In short, these amino acids serve as markers for hydroxyl radical activity.

Inspection of Table 2 shows various peptide fragments that contain amino acids which are known to react with hydroxyl radicals. We present the following brief discussion of several amino acids.

Tyrosine

The amino acid tyrosine (Y) is present in both the *T. rex* and *B. canadensis* histone protein fragments (Table 2). This particular amino acid has been the subject of extensive study and found to readily react with active oxygen species. Bartesaghi et al. (2010) show that tyrosine is sensitive to oxidative alteration and C-C bond formation resulting in cross-linking:

> Tyrosine dimerization and nitration to 3,3´-dityrosine and 3-nitrotyrosine (3-nitro-Tyr), respectively, represent biologically-relevant oxidative post-translational modifications in proteins generated by the reactions with reactive oxygen and nitrogen intermediates both in vitro and in vivo. These tyrosine oxidation processes depend on the intermediate formation of tyrosyl radical (•Tyr), a transient species formed by the one-electron oxidation of tyrosine. For instance, 3,3´-dityrosine formation results from the termination reaction between two •Tyr radicals with the formation of a new C-C bond; 3,3´-dityrosine participates in protein cross-linking (1) and also serves as a marker for oxidatively damaged proteins. Indeed, elevated levels of 3,3´-dityrosine can be found as a product of aging, inflammation, exposure to UV and γ-radiation and other oxidative stress conditions. (Bartesaghi et al., 2010, p. 822)

If iron as Fe^{2+} is driving formation of HO• and fixing or cross-linking peptide fragments, it is reasonable to assume that the tyrosine marker would have registered the presence of HO• perhaps in the form of a cross-linked species. Tyrosine was unaltered in all of the histone fragments (Table 2). It would seem if a significant amount of hydroxyl radical was present tyrosine would have reacted as well.

Schweitzer's model hypothesizes that hydroxyl radical formed by Fe^{2+} fixes proteins through cross-linking, but fails to account for the presence of unaltered tyrosine, which is sensitive to oxidative stress. The point is that even if Fenton chemistry had prevailed, it is unlikely that all of the tyrosine side chains would have survived for 68 million years.

Methionine

One register that is sensitive to the presence hydroxyl radical is methionine. Schweitzer found the sulfur containing amino acid in a histone peptide (M[+16]) (Peptide 17, Histone 4, TVTAM[+16]DVVYALK, *B. canadensis*,

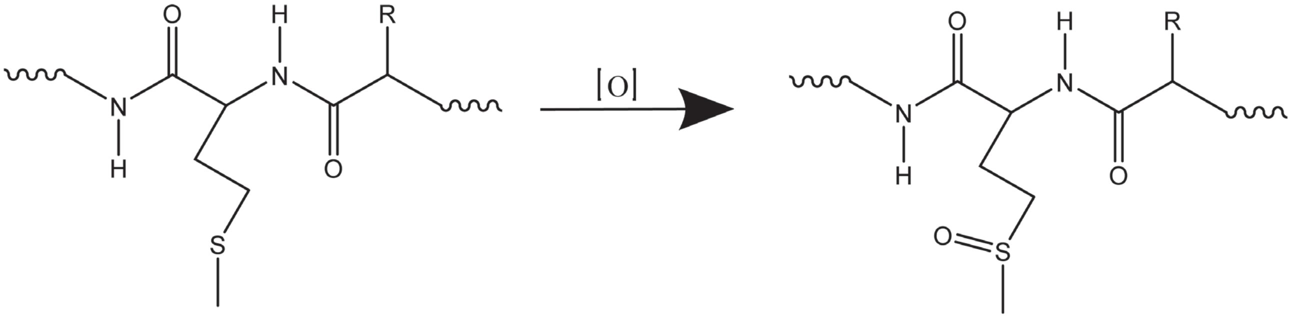


Figure 5. Oxidation of methionine residue to methionine sulfoxide.

Table 2). Methionine was lightly oxidized which Schweitzer et al reported was an "*in vitro* artifact." This is generally taken to mean that oxidation happened *upon handling* of the sample during work up. From this we can infer that the thioether group remained unoxidized during its long entombment while other parts of the same polypeptide were cross-linked—if the Fenton chemistry proposal is assumed. A closer inspection of this proposal reveals problems concerning the oxidative stability of the methionine residue (Figure 5).

Firstly, it is acknowledged that methionine does oxidize upon handling. For example, Hongcheng et al. (2013) discusses the oxidation sensitivity of methionine during laboratory handling.

> Methionine (Met) oxidation is a major modification of proteins, which converts Met to Met sulfoxide as the common product. It is challenging to detect the level of Met sulfoxide, because it can be generated *during sample preparation* and analysis as an artifact. (Hongcheng et al., 2013, p. 11705; emphasis added)

Schweitzer is correct to suggest that the methionine residue might suffer *in vitro* alteration. Yet the consequence of this suggestion is hardly insignificant. Did methionine survive 68 million years in the ground unaltered while in the presence of hydroxyl radicals? Given the great proposed antiquity of this peptide fragment and the proposed presence of hydroxyl radicals, it is surprising that the fully oxidized product was not detected.

Researchers have observed that larger suites of oxidized products are to be expected even for younger specimens (Hiller et al., 1981; Bobrowski et al., 1987; Bobrowski and Holcman, 1989; Bobrowski et al., 1991; Schöneich et al., 1994). Parker et al. (2011, p. 212) report, "methionine decomposes readily in the presence of oxygen and produces methionine sulfoxide, methionine sulfone, and various sulfides and thiols."

Bern et al. (2010) studied the reaction of a model polypeptide (Gly-Met-Gly) in the presence of flash photo generated *hydroxyl radical*. The group reports that "this oxidation method produces diffusing hydroxyl radicals"(Bern et al., 2010, p. 3). The method altered the methionine residue producing oxidized side chains (Figure 6).

Methionine is not, it would seem stable in the presence of hydroxyl free radicals; yet to be clear, Schweitzer suggests,

> Through a cascade of events referred to as Fenton chemistry, highly reactive hydroxyl radicals triggered both cross-linking of proteins and peroxidation and crosslinking of proteins and peroxidation and crosslinking of fatty acids making up cell membranes. (Schweitzer, 2013b, p. 421)

Did methionine avoid oxidative assault by the "highly reactive hydroxyl radical" for some fraction or all of 68 million years while other parts of the peptide fragments were successfully and selectively cross-linked by the same hydroxyl radicals? The proposal that the oxidation-prone methionine residue can survive 68 million years through aggressive environmental weather changes and fugitive free radical assault and then end its career through *laboratory* handling in its mildly oxidized form (sulfoxide), appears to be a just so story. A reasonable expectation is that methionine should have been oxidized in the presence of fugitive hydroxyl radicals resulting in its homocysteine form or at least its sulfone form at some time in its long burial period. Homocysteine or methionine sulfone was undetected.

Histidine

Another amino acid that is a "litmus test" for hydroxyl radical presence is histidine. This residue has been described as an effective *antioxidant*.

> Experimental data show that the antioxidant effect of these endogenous substances is likely to be provided by histidine residue. In fact, in its free state this amino-acid is a HO-radical catcher and one of the most effective "suppressors" of singlet oxygen. …Indeed oxidation of histidine to 2-oxohistidine is a good marker of

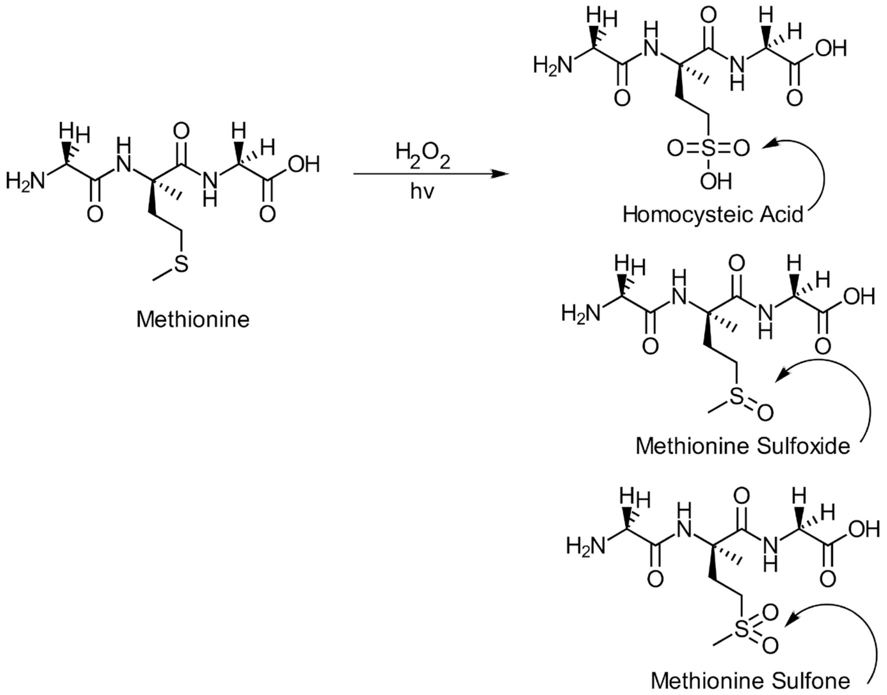


Figure 6. Flash photo-generated hydroxyl radical treatment of Gly-Met-Gly.

> oxidative stress. (Shleikin and Medvedev, 2014, p. 125)

Farber and Levine (1986, p. 4577), more specifically report:

> Oxidative modification of susceptible enzymes proceeds under anaerobic conditions in the presence of Fe^{2+} and H_2O_2. Each mixed function oxidation system examined thus far can reduce Fe^{3+} to Fe^{2+} and, in the presence of oxygen, can generate H_2O_2. A simple summary of the reaction is as follows.

$$Fe^{2+} + \text{enzyme} \rightarrow Fe^{2+}\text{- enzyme}$$

$$Fe^{2+}\text{- enzyme} + H_2O_2 \rightarrow \text{enzyme-[O]} + Fe^{3+}$$

The ferric iron can again be reduced to the ferrous form and can catalyze the oxidation of additional protein molecules.

The group notes that oxidation of an enzyme proceeds in a site-specific fashion resulting in the oxidation of particular histidine residues.

Zhao et al. (1997, p. 9028) studying a copper oxidizing system analogous to the ferrous/ferric oxidizing system notes the following:

> We note that the formation of 2-oxo-His via the displayed pathway requires the net addition of HO· at C-2 of the imidazole ring whereas ESR investigations on the addition of radiation chemically produced freely diffusable hydroxyl radicals to imidazole derivatives not complexed to transition metals have provided evidence for the addition of HO· at C-4 and C-5.

Figure 7 provides an illustration of this histidine oxidation.

While some distinction is made by Zhao et al. (1997) concerning the source and subsequent position of hydroxyl radical addition, it is clear that the presence of hydroxyl radicals leads to ring oxidation. Assuming, freely diffusible hydroxyl radicals have saturated the various protein fragments resulting in, according to Schweitzer, fixing or cross-linking the protein, one would expect oxidized histidine, yet none was reported. The absence of oxidative alteration of these sensitive markers Tyr (Y), Met (M), His (H)) is not easily explainable, assuming Fenton chemistry operated during the deep time burial period of the dinosaurian specimens.

Radiocarbon Dating

We lastly note perhaps the most disappointing absence in Dr. Schweitzer's analysis. Assuming that the protein substances discussed are indeed dinosaurian soft tissue, it is also true that the C,H,N,O elemental makeup of the proteins are endogenous. As such, the samples are highly qualified for a carbon-14 analysis. C-14 should help further the discussion on the possible age of the organic material. If this genuine protein matter is 68 million years old, C-14 should be absent. If the tissue is 50,000 years or younger, C-14 will be detected. We also note in passing that C-14 presence if found and not interpreted as a young age must be interpreted as contamination making unreliable the mass spec data presented by Schweitzer.

Radiocarbon dates that have been reported on various dinosaur samples, which all consistently fall within ranges of about 18,000–30,000 years before present are not regarded as reliable, because dinosaurs are believed to be extinct for some tens of millions of years (see Thomas and Nelson, 2015, for a detailed analysis of C-14 in dinosaur fossils). It is equally interesting that Dr. Schweitzer has not subjected her soft tissue dinosaur samples to radiocarbon dating. To our knowledge, Schweitzer's writings have not offered any explanation for this omission.

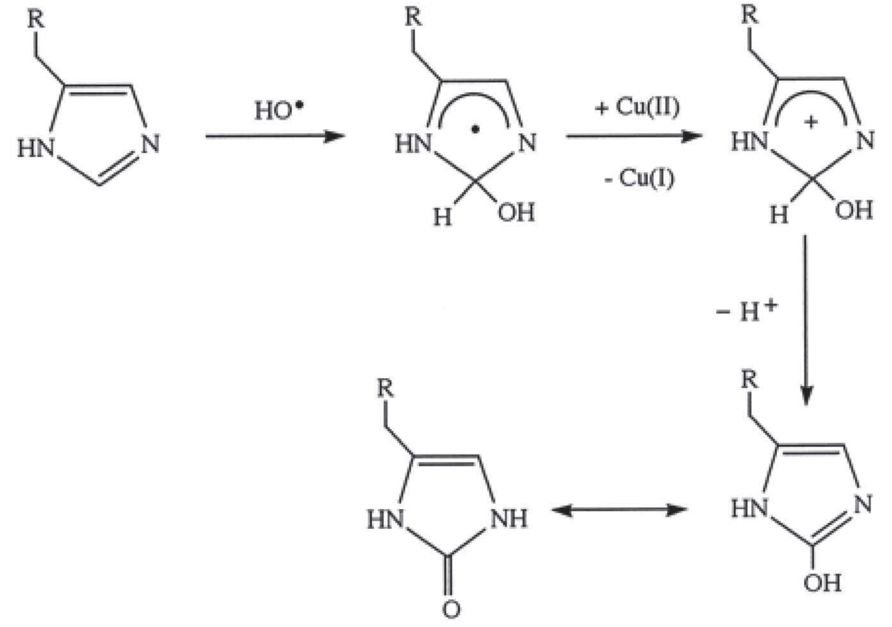


Figure 7. Oxidation of His residue to 2-oxo-His via Cu(II) (Zhao et al. 1997)

Conclusion

Schweitzer has hypothesized that molecular sheltering and lipophilic amino acid character impart deep time survival to protein fragments recovered in *T. rex* and *B. canadensis*. She also invokes Fenton type chemistry to explain protein cross-linking and consequently deep time survival. Some of these motifs perhaps played a role to account for soft-tissue survival, but they seem inadequate to explain deep-time survival—if model studies are to be trusted at all. These studies predict that hydrolytically unstable residues such as asparagine, and glutamine perhaps metaphorically referred to as molecular clocks should have "run down" to their carboxylic acid analogues or altered their native peptide chains long ago. Tantalizingly, the presence of aspartic acid suggests hydrolysis of asparagines, but the absence of isoaspartic acid suggests the aspartic acid residues may be native to the sequence and not the product of asparagines hydrolysis. Glutamine also appears in the peptide fragments in addition to its hydrolyzed analogue (glutamic acid). In one instance the two appear side by side, again frustrating a simple trend or pattern of hydrolysis.

While degradation may not be uniform in a field setting and we may well see advanced decomposition for some remains and lagging decomposition in other remains, it is difficult to explain how hydrolytically sensitive moieties escaped aqueous and intramolecular transformations over vast epochs of time. In this same way, if free radical generation proceeded as suggested through aqueous Fenton type chemistry to cross-link peptides, it would seem tyrosine, methionine and histidine would have been oxidized sometime during the long burial period. Is it reasonable to invoke hydroxyl radical fixation for the bulk polypeptide matrix while having no registry of this phenomenon in several sensitive hydroxyl radical markers? It is readily admitted that a stabilization mechanism is at work buffering hydrolysis, suppressing neighboring group attack and quenching free radical degradation. It is not so clear that Schweitzer has unambiguously identified those mechanisms since chemical markers are present that militate against her immediate proposal.

We propose that one preservative mechanism has been overlooked and may be the most obvious of all. It is perhaps found in a helpful maxim coined here; soft tissue survival is tied to soft tissue age. We believe that the soft tissue elucidated by Schweitzer et al. (2005a, 2007, 2013a, 2013b) was not buried for 68 million years but some vastly shorter period. This surprisingly simple explanation when combined with some of the other preservation motifs, potentially favors survival of asparagine, glutamine, tyrosine, methionine and histidine. This hypothesis is also favorable to model studies that predict short age survival of peptides, polynucleic acids and as cited within this text the discovery of C-14 in fossil specimens—which might be present in Schweitzer's specimens. *If in fact the dinosaurian specimens are not old, soft tissue remains may even be expected to be provided a kind of short term temporal sheltering in contradistinction to long term temporal assault from prolonged exposure to degradative elements such hydrolysis, free radical attack, and elevated temperature.*

Thus, we raise the possibility here that the survival of collagen and osteocyte peptide fragments is parsimoniously explained by a young age hypothesis which envisages far fewer years of degradative stress and improves the chances of survival of sensitive chemical markers. The young age hypothesis, while counterintuitive to conventional thought concerning the age of the dinosaurs, is unavoidably visible for all to consider. Indeed it may well turn out that soft tissue survival is tied to soft tissue age, and these dinosaur remnants are among the first of many more and even better preserved specimens that lay undiscovered in the museums and laboratories across the world.

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References

Anderson, K. 2015. Dinosaur tissue or bacterial biofilms? *Creation Research Society Quarterly* 51:259–267.

Armitage, M.H., and K.L. Anderson. 2013. Soft sheets of fibrillar bone from a fossil of the supraorbital horn of the dinosaur *Triceratops horridus*. *Acta Histochemica* 115:603–608.

Associated Press. 2012. Scientists recover T. rex soft tissue. http://www.nbcnews.com/id/7285683/ (accessed Nov. 8, 2013)

Avanti, C. 2012. *Innovative Strategies for Stabilization of Therapeutic Peptides in Aqueous*

Formulations. https://www.tipharma.com/fileadmin/user_upload/Theses/PDF/Christina_Avanti_D6–202.pdf

Barb, W.G., J.H. Baxendale, P. George, and K.R. Hargrave. 1951. Reactions of ferrous and ferric ions with hydrogen peroxide. Part 1, the ferrous ion question. *Transactions of the Faraday Society* 47:462–500.

Bartesaghi, S., J. Wenzel, M. Trujillo, M. Lopez, J. Jospeh, B. Kalyanaraman, and R. Radi. 2010. Lipid peroxyl radicals mediate tyrosine dimerization and nitration in membranes. *Chemical Research in Toxicology* 23(4): 821–835.

Bern M., J. Saladino, and J. Sharp. 2010. Conversion of methionine to homocysteic acid in heavily oxidized proteomics samples. *Rapid Communications in Mass Spectrometry* 24(6): 768–772.

Bobrowski K., and J. Holcman. 1989. Formation and stability of intramolecular three-electron SN, SS, and SO bonds in one-electron-oxidized simple methionine peptides. Pulse radiolysis. *Journal of Physical Chemistry* 93:6381–6387.

Bobrowski, K., K.L. Wierzchowski, J. Holcman, and M. Ciurak. 1987. Intramolecular charge-transfer between tryptophan and tyrosine in peptides with bridging prolines. *Studies in Biophysics* 122:23.

Bobrowski K., C. Schöneich, J. Holcman, and K.-D. Asmus. 1991. •OH radical induced decarboxylation of methionine-containing peptides. Influence of peptide sequence and net charge. *Journal of the Chemical Society Perkin Transactions* 2:353–362.

Buckley, M., A. Walker, S.Y.W. Ho, Y. Yang, C. Smith, P. Ashton, J.T. Oates, E. Cappellini, H. Koon, K. Penkman, B. Elsworth, D. Ashford, C. Solazzo, P. Andrews, J. Strahler, B. Shapiro, P. Ostrom, H. Gandhi, W. Miller, B. Raney, M.I. Zylber, M.T.P. Gilbert, R.V. Prigodich, M. Ryan, K.F. Rijsdijk, A. Janoo, and M.J. Collins. 2008. Comment on "protein sequences from Mastodon and *Tyrannosaurus rex* revealed by mass spectrometry." *Science* 319:33.

Cappellini, E., L.J. Jensen, D. Szklarczyk, A. Ginolhac, R.A.R. da Fonseca, T.W. Stafford, S.R. Holen, M.J. Collins, L. Orlando, E. Willerslev, M.T. Gilbert, and J.V. Olsen. 2012. Proteomic analysis of a pleistocene mammoth femur reveals more than one hundred ancient bone proteins. *Journal of Proteome Research* 11:917–926.

Catak, S., G. Monard, V. Aviyente, and M.F. Ruiz-Lopez. 2009. Deamidation of asparagine residues: direct hydrolysis versus succinimide-mediated deamidation mechanisms. *Journal of Physical Chemistry* A 113:1111–1120.

Cleland, T.P., K. Voegele, and M.H. Schweitzer. 2012. Empirical evaluation of bone extraction protocols. *PLoS ONE* 7(2): e31443.

Cody, G.D., N.S. Gupta1, D.E.G. Briggs, A.L.D. Kilcoyne, R.E. Summons, F. Kenig, R.E. Plotnick, and A.C. Scott. 2011. Molecular signature of chitin-protein complex in Paleozoic arthropods. *Geology* 39:255–258.

Cournoyer, J.J., J.L. Pittman, V.B. Ivleva, E. Fallows, L. Waskell, C.E. Costello, and P.B. O'Connor. 2005. Deamidation: differentiation of aspartyl from isoaspartyl products in peptides by electron capture dissociation. *Protein Science* 14:452–463.

Crucefix, L. 2013. World's oldest dinosaur embryo bonebed yields organic remains. http://www.utm.utoronto.ca/main-news-research-news-general/worlds-oldest-dinosaur-embryo-bonebed-yields-organic-remains (accessed August 18, 2014).

Dizdaroglu, M., and E. Gajewski. 1989. Structure and mechanism of hydroxyl radical-induced formation of a DNA-protein cross-link involving thymine and lysine in nucleohistone. *Cancer Research* 49:3463–3467.

Edwards, N.P., H.E. Barden, B.E. van Dongen, P.L. Manning, P.L. Larson, U. Bergmann, W.I. Sellers, and R.A. Wogelius. 2011. Infrared mapping resolves soft tissue preservation in 50 million year-old reptile skin. *Proceedings of the Royal Society B* 278:3209–3218.

Farber, J.M., and R.L. Levine. 1986. Sequence of a peptide susceptible to mixed-function oxidation probably cation binding site. Probable cation binding site in glutamine synthetase. *The Journal of Biological Chemistry* 261:4574–4578.

Fields, H. 2006. Dinosaur shocker. *Smithsonian Magazine*. http://www.smithsonianmag.com/science-nature/dinosaur.html?c=y&page=2)

Glass, K., S. Ito, P.R. Wilby, T. Sota, A. Nakamura, C.R. Bowers, J. Vinther, S. Dutta, R. Summons, D.E.G. Briggs, K. Wakamatsu, and J.D. Simon. 2012. Direct chemical evidence for eumelanin pigment from the Jurassic period. *Proceedings of the National Academy of Sciences, USA* 109:10218–10223.

Hawkins, C.L., and M.J. Davies. 2001. Generation and propagation of radical reactions on proteins. *Biochemica and*

Biophysica Acta 1504:196–219.

Hedges, R.E.M. 2002. Bone diagenesis: an overview of processes. *Archaeometry* 44:3:319–328.

Hiller K.-O., B. Masloch, M. Göebl, and K.-D. Asmus. 1981. Mechanism of the hydroxyl radical induced oxidation of methionine in aqueous solution. *Journal of the American Chemical Society* 103:2734–2743.

Hongcheng, L., G. Ponniah, A. Neill, R. Patel, and B. Andrien. 2013. Accurate determination of protein methionine oxidation by stable isotope labeling and LC-MS analysis. *Analytic Chemistry* 85:11705–11709.

Kaye, T.G., G. Gaugler, and Z. Sawlowicz. 2008. Dinosaurian soft tissues interpreted as bacterial biofilms. PLoS ONE 3(7): e2808.

Klotz, A.V., and B.A. Thomas. 1993. N5-Methylasparagine and asparagines as nucleophiles in peptides: main-chain vs. side-chain amide cleavage. *Journal of Organic Chemistry* 58:6985–6989.

Lindgren, J., P. Uvdal, A. Engdahl, A.H. Lee, C. Alwark, K.-E. Bergquist, E. Nilsson, P. Kström, M. Rasmussen, D.A. Douglas, M.J. Polcyn, and L.L. Jacobs. 2011. Microspectroscopic evidence of cretaceous bone proteins. *PloS One* 6:4:e19445.

Lindqvist, C., S.C. Schuster, Y. Sun, S.L. Talbot, J. Qi, A. Ratan, L.P. Tomsho, L. Kasson, E. Zeyl, J. Aars, W. Miller, Ó. Ingólfsson, L. Bachmann, Ø Wiig. 2010. Complete mitochondrial genome of a Pleistocene jawbone unveils the origin of polar bear. *Proceedings of the National Academy of Sciences USA* 107:5053–5057

Liu, H., G. Ponniah, A, Neill, R. Patel, and B. Andrien. 2013. Accurate determination of protein methionine oxidation by stable isotope labeling and LC-MS analysis. *Analytical Chemistry* 85:24:11705–11709.

Parker, E., H.J. Cleaves, M.P. Callahan, J.P. Dworkin, D.P. Glavin, A. Lazcano, J.L. Bada. 2011. Prebiotic synthesis of methionine and other sulfur-containing organic compounds on the primitive earth: a contemporary reassessment based on an unpublished 1958 Stanley Miller experiment. *Origins of Life and Evolution of Biospheres* 41(3): 201–212.

Pawlicki, R. 1977. Histochemical reactions for mucopolysaccharide in the dinosaur bone. *Acta Histochemica* 58:75–78.

Pawlicki, R., A. Korbel, and H. Kubiak. 1966. Cells, collagen fibrils and vessels in dinosaur bone. *Nature* 211:655–657.

Pawlicki, R., and M. Nowogrodzka-Zagórska. 1998. Blood vessels and red blood cells preserved in dinosaur bones. *Annals of Anatomy* 180:73–77.

Payne, R.W., and M.C. Manning. 2009. Peptide formulation: challenges and strategies. *Innovations in Pharmaceutical Technology* 28:64–68.

Peake, T. 2012. Analysis of dinosaur bone cells confirms ancient protein preservation. http://news.ncsu.edu/releases/tpschweitzer-bone (accessed August 17, 2014).

Pevzner, P.A., S. Kim, and J. Ng. 2008. Comment on protein sequences from mastodon and *Tyrannosaurus rex* revealed by mass spectrometry. *Science* 321:1040.

Prousek, J. 2007. Fenton chemistry in biology and in medicine. *Pure & Applied Chemistry* 29:2325–2338.

Reisz, R.R., D.C. Evans, E.M. Roberts, H-D. Sues, and A.M. Yates. 2012. Oldest known dinosaurian nesting site and reproductive biology of the Early Jurassic sauropodomorph Massospondylus. *Proceedings of the National Academy of Sciences USA* 109:2428–2433.

Reisz, R.R., T.D. Huang, E.M. Roberts, S. Peng, C. Sullivan, K. Stein, A.R.H. LeBlanc, D. Shieh, R. Chang, C. Chiang, C. Yang, and S. Zhong. 2013. Embryology of early Jurassic dinosaur from China with evidence of preserved organic remains. *Nature* 496:210–214.

Robinson, A.B., and L.R. Robinson. 1991. Distribution of glutamine and asparagines residues and their near neighbors in peptides and proteins. *Proceedings of the National Academy of Sciences USA.* 88:8880–8884.

San Antonio, J.D., M.H. Schweitzer, S.T. Jensen, R. Kalluri, M. Buckley, J.P.R.O. Orgel. 2011. Dinosaur peptide suggest mechanisms of protein survival. *PLoS One* 6:e20381.

Sang-aroon, W., and V. Ruangpornvisuti. 2013. Theoretical study on isomerization and peptide bond cleavage at aspartic residue. *Journal of Molecular Modeling* 19:3627–3636.

Schöneich C., F. Zhao, K.P. Madden, and K. Bobrowski. 1994. Side chain fragmentation of N-terminal threonine or serine residue induced through intramolecular proton transfer to hydroxyl sulfuranyl radical formed at neighboring methionine in dipeptides. *Journal of the American Chemical Society* 116:4641–4652.

Schweitzer, M.H., M. Marhsall, K. Carron, D.S. Bohle, S.C. Busse, E.V. Arnold, D. Barnard, J.R. Horner, and J.R. Starkey. 1997. Heme compounds in dinosaur trabecular bone. *Proceedings of the National Academy of Sciences USA* 94:6291–6296.

Schweitzer, M., J.L. Wittmeyer, J.R. Horner, and J.K. Toporski. 2005a. Soft-tissue vessels and cellular preservation in *Tyrannosaurus rex*. *Science* 307:1952–1955.

Schweitzer, M.H., L. Chiappe, A.C. Garrido, J.M. Lowenstein, and S.H. Pincus. 2005b. Molecular preservation in Late Cretaceous sauropod dinosaur eggshells. *Proceedings of the Royal Society B* 272:775–784.

Schweitzer, M.H., J.L. Wittmeyer, and J.R. Horner. 2007. Soft tissue and cellular preservation in vertebrate skeletal elements from the Cretaceous to the present. *Proceedings of the Royal Society B* 274:183–197.

Schweitzer, M.H. 2010. Blood from stone. *Scientific American* 303(6): 62–69.

Schweitzer, M.H., W. Zheng, T.P. Cleland, M.B. Goodwin, E. Boatman, E. Theil, M.A. Marcus, and S.C. Fakra. 2013a. A role for iron and oxygen chemistry in preserving soft tissues, cells and molecules from deep time. *Proceedings of the Royal Society B* 281:20132741.

Schweitzer, M.H., W. Zheng, T.P. Cleland, M. Bern. 2013b. Molecular analyses of dinosaur osteocytes support the presence of endogenous molecules. *Bone* 52:414–423.

Shleikin, A.G., and Y.V. Medvedev. 2014. Role of peroxidation and heme catalysis in coloration of raw meat. *Scientiarum Polonorum Technologia Alimentaria* 13(2): 123–127. http://www.food.actapol.net/pub/1_2_2014.pdf.

Thomas, B., and V. Nelson. 2015. Radiocarbon in dinosaur and other fossils. *Creation Research Society Quarterly* 51:299–311.

Zhao, F., E. Ghezzo-Schöneich, G.I. Aced, J. Hong, T. Milby, and C. Schöneich. 1997. Metal-catalyzed oxidation of histidine in human growth hormone mechanism, isotope effects, and inhibition by a mild denaturing alcohol. *Journal of Biological Chemistry* 272:14:9019–9029.

Creation Research Society Quarterly 2015. 51:286–298.

The Hell Creek Formation: The Last Gasp of the Pre-Flood Dinosaurs

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Abstract

According to secularists, the top of the Hell Creek Formation records the last of five great extinctions. It has gained further fame as a unit that documents the disappearance of dinosaurs in the Western United States. The so-called Cretaceous-Paleogene (K-Pg) extinction is more complicated than many are led to believe, and is probably just the last appearance of many organisms in Flood-deposited strata. The Hell Creek Formation extends across eastern Montana and parts of North and South Dakota, varying from 170 m to 41 m thick. The base of the formation is picked where the composition of the underlying sandstone layer (Fox Hills Formation) changes to carbonaceous sandstone, marking the lowermost bed of the Hell Creek Formation. The top of the unit is more complicated. In the past, changes in the stratigraphy and/or fossils were used to pick the upper boundary, but now secularists choose the Iridium-rich layer as the top, regardless of other geological data. Limited occurrences of an Iridium anomaly make picking this top problematic in many locations. The type section shows ample evidence of water deposition. Marine fossils, including sharks, bivalves, and gastropods, are prevalent throughout the Hell Creek, not just in isolated lenses as many have claimed. Patterns of dinosaur occurrences in the uppermost Hell Creek show less dinosaur fossils toward the top of the formation and a 2–3 m gap at the very top that is devoid of dinosaur fossils. Dinosaur fossils found in the overlying lowermost Paleocene Fort Union Formation, may indicate some dinosaurs survived until the end of the Zuni Megasequence, slightly above the K-Pg. All geological data observed in the Hell Creek Formation are interpreted as occurring during a worldwide Flood event. Stratigraphic data, such as ripples and cross-bedded sandstones, demonstrate water transport. Marine fossils found throughout the formation imply a strong marine influence during deposition of the entire unit. The observed mixing of land and sea organisms is best explained by tsunami-like waves transporting ocean waters onto the continent, engulfing the terrestrial animals and depositing the Hell Creek Formation.

Introduction

The Hell Creek Formation is probably most famous as the unit that has produced the majority of *Tyrannosaurus rex* specimens. It is now becoming even more famous for its production of dinosaur soft-tissue fossils, which exhibit remarkable preservation, demonstrating that these fossils cannot be millions of years old as claimed by secularists (e.g., see Thomas, 2015). The formation is also extremely important in the secular dinosaur extinction debate. It is one of the only units in the world that secular scientists believe contains significant numbers of dinosaur fossils from rocks from the very end of the Cretaceous

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system (Lucas, 2007). In addition, it is claimed the formation provides the most detailed history of continental biotic changes across the K-Pg (Cretaceous-Paleogene) boundary in the world (Clemens, 2002; Clemens and Hartman, 2014). Yet, the top of the Hell Creek also contains the famous "3 m gap," where no dinosaur specimens have been discovered (Johnson et al., 2002).

The Hell Creek Formation is one of the last deposits of the Zuni megasequence across the northern Great Plains region. Megasequences are defined as packages of sedimentary rock bounded top and bottom by erosional surfaces, with coarse sandstone layers at the bottom (deposited first), followed by shales, and then limestone at the top (deposited last) (Sloss, 1963). The corresponding size of the sedimentary particles is also thought to decrease upwardly in each megasequence. The megasequences are interpreted by secular geologists as representative of the depth of the sea at the particular time each one was deposited. The base sandstone layers of each megasequence are believed to represent the shallowest sea level, the shale representing a little deeper water environment, and the limestone the deepest water environment in each sequence.

Primarily described as a siliciclastic rock unit, the Hell Creek Formation has been labeled by secular geologists as a Late Cretaceous depositional event. Above this unit are Paleogene (Tertiary) system rocks of the Fort Union Formation (Tullock Member), the basal unit of the Tejas megasequence (Blakey, 2010). The Hell Creek Formation extends 700 km across eastern Montana and North Dakota, and an approximately equal distance from northeastern Wyoming (where it is equivalently called the Lance Formation) to the Canadian border (where it is called the Frenchman Formation) (Figure 1). The formation's best exposures are in eastern Montana and North and South Dakota, along the outer margins of the Williston Basin (Figure 1).

K-Pg: Just One of Five Great Extinctions?

Secularists claim to have identified five so-called great extinctions in the Phanerozoic Eon, one each in the Late Ordovician, the Late Devonian, the Permian-Triassic, the Late Triassic, and, finally, the end Cretaceous or K-T (now K-Pg) event. Table 1 summarizes the secular view of these five events by the percent extinction per species and by vertebrate extinction rate. Looking at only vertebrate extinction through time, by families per million years, we see the same five events, but the relative family extinction rates are different from the overall species extinction percent. This demonstrates some of the statistical bias that can be used, depending on whether the focus is on extinctions by species or families or by rates of extinctions versus extinction percent.

One of the great secular mysteries of all time concerns the extinction of dinosaurs at the end of the Cretaceous System, of which the Hell Creek Formation plays a major part. This extinction

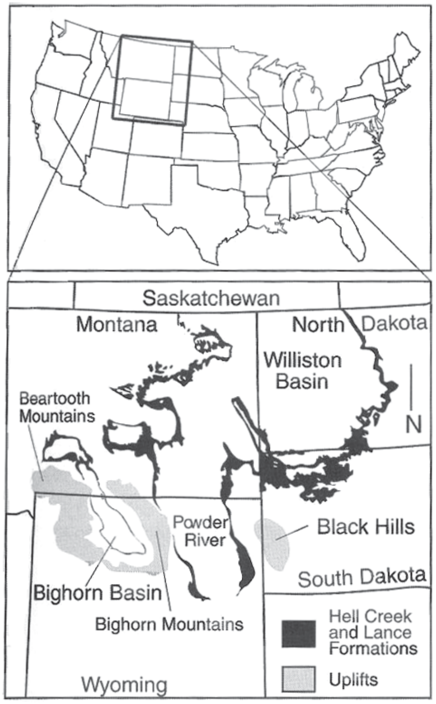


Figure 1. Location map of the Hell Creek Formation and the equivalent Lance Formation. Outcrop area is shown in black. The Hell Creek Formation extends under the Williston Basin in eastern Montana and North Dakota. In the Powder River and Bighorn Basins, it is known as the Lance Formation. Modified from Hicks et al. (2002).

Extinction Event	Species Extinction (%)	Vertebrate Extinction Rate (Families per Million Years)	Secular Age (Ma)
Cretaceous-Paleogene	70	16	65.9
Upper Triassic	76	10	206
Permian-Triassic	96	15	248
Upper Devonian	82	10	354
Upper Ordovician	85	20	443

Table 1. The so-called five great extinctions in the sedimentary record, showing purported extinctions by species and vertebrate extinction rate, assuming the secular ages listed. All ages are listed in millions of years.

is commonly called the "K-T" or "K-Pg" event because it occurred at the Cretaceous-Tertiary (Paleogene) boundary. The general secular consensus on the date of the K-Pg extinction event was approximately 65 million years before present. However, this date has been recently revised to 65.95 million years (Kuiper et al., 2008).

There have been over 65 serious published ideas attempting to explain this extinction (Benton, 1990). Some of these ideas, as summarized by Powell (1998), include medical problems, overspecialization, competition with mammals, plant changes, climate changes, sea-level changes, increased volcanism, and extraterrestrial events. The most popular theory currently appears to be the asteroid impact.

The asteroid theory is based on the "accidental" discovery of higher-than-expected levels of iridium (Ir) within the K-Pg boundary layer at Gubbio, Italy (Alvarez et al., 1980). Similarly high levels of Ir were subsequently found at many sites worldwide at the K-Pg boundary. Iridium is an element found in meteorites but is not very common on Earth, and so it is thought to have come from space. Many have concluded that this Ir-rich layer was deposited after an asteroidlike body struck Earth, scattering Ir-rich dust all over the globe.

Further study of the K-Pg boundary layer found tiny crystals of "shocked" quartz at many of these same locations (Bohor et al., 1984). Shocked quartz can form only under extremely high-pressure conditions, like impact sites, further strengthening the evidence for an impact at the K-Pg. In 1992, microscopic diamonds were reported from the K-T layer in Alberta, Canada (Carlisle, 1992). It is thought that the diamonds are fragments of impacts, as they are "cleaner" and contain less nitrogen than traditional mantle-derived diamonds.

The presumed site of the K-Pg impact was located near the northwestern edge of the Yucatan Peninsula, Mexico (Hildebrand et al., 1991). The site consists of a large, circular gravity anomaly known as the Chicxulub Crater. It is 110 miles (180 km) in diameter, allowing room for a 6-mile (10 km) wide asteroid to have created the dimensions of the crater. There is, however, disagreement on the exact size of the crater.

However, many dinosaur paleontologists do not feel an asteroid impact is the answer to this mystery (Keller et al., 2007; Keller and Adatte, 2007). They believe an impact happened but that it had only a minimal extinction effect. Dr. Robert Bakker (one of America's leading dinosaur paleontologists and author of *The Dinosaur Heresies*) believes dinosaurs went extinct from an epidemic brought on by a rapid drop in sea level. He thinks dinosaurs were killed by foreign diseases carried from continent to continent across land bridges.

Mr. Jack (John R.) Horner, noted dinosaur specialist and professor of paleontology at Montana State University, spoke about extinction at a lecture at Central Michigan University in April 2001. He stated that the meteor or asteroid theory of extinction is a "good example of taking an idea too far," noting that this theory is all based on negative evidence. He pointed out that "just a few dinosaur taxa (3–4) were living at the end, or close to the K-T [Pg] boundary," and that there is "no record of any catastrophic event that correlates with the last of the dinosaurs." Horner frequently reminds his audiences that no dinosaur bones have ever been found within 3 m of the K-Pg boundary, supporting the idea that dinosaurs were extinct prior to the deposition of the Tertiary System. He believes that dinosaurs went extinct from simultaneous sea-level changes and climatic shifts. He readily admitted, "I don't care. I'm just glad they're gone. I want to go out for walks at night."

Many "extinctions" seem to coincide with simultaneous rapid change in rock type and/or depositional pattern at or near a particular rock boundary. These rapid changes in rock type would seem to indicate condition changes that could rapidly "finish off" a population, like the dinosaurs in the Hell Creek Formation and elsewhere. Rapid shifts in depositional patterns are an expected consequence of a global Flood.

A review of 110 secular research papers on the K-Pg extinction, examining all organisms, showed that a sudden extinction was noted in only 50% of the papers, and that 72% of those sudden extinctions were associated with a corresponding rapid shift in rock type (Clarey and Heim, 2012). This strong correlation demonstrated that the perceived abruptness of the K-Pg extinction may be enhanced by observable changes in the rocks. This would be predicted as Flood sediments were being piled on top of one another, accumulating new sediments in rapid succession, with new organisms appearing and disappearing just as abruptly. Clarey and Heim (2012) also found that 28% of the so-called extinctions occurred prior to the K-Pg boundary, 55% occurred at the K-Pg boundary, and 17% occurred just after the K-Pg boundary, in the Early Tertiary system. Rather surprisingly, only 57% of the papers reported an iridium anomaly present in the boundary layer. So, it appears there is no definitive stratigraphic boundary where all organisms, including the dinosaurs, went extinct. Organisms merely stopped being deposited at those locations.

There are no extinctions as described by secular science. Extinction in the rock record is defined as the highest stratigraphic occurrence of a fossilized organism. During the Flood, layers of animal and plant communities were rapidly piled on top of one another, from various directions and in seemingly a global order, according to the tectonic activity taking place at that moment. Viewed in this manner, these last occurrences are not extinctions. Instead, they are merely the last record of various organisms trapped by the Floodwaters. Some creation scientists think the "K-Pg" was about the high point of the Floodwaters, when all land animals finally succumbed and drowned. That may be why at this point so many organisms seemed to simultaneously go "extinct." It was a major juncture in the Flood record, and the Hell Creek Formation has recorded it.

Stratigraphy of the Hell Creek Formation

The Hell Creek Formation was first named and described by Brown (1907), who separated it from the underlying Fox Hills Formation and the overlying Fort Union Formation, although he failed to identify a type section in his description (Hartman, 2002). Thickness varies from 170 m in Garfield County, Montana, to only 41 m in McCone County, Montana (Johnson et al., 2002). More commonly, the unit varies between 60–100 m in thickness (Johnson et al., 2002).

Base of the Formation

Some authors claim the base of the formation to be a regional unconformity surface, as it contains cobbles up to 10 cm in diameter (Bauer, 1925; Jensen and Varnes, 1964) and lacks lignite beds, which are thought to represent a sea-level regression sequence (Johnson et al., 2002). However, there is no physical evidence of erosion at the basal boundary. It merely changes from white sandstone at the top of the underlying Fox Hills Formation (Colgate Member) to a 0.3–1 m carbonaceous sandstone that is remarkably persistent across southern North Dakota (Murphy et al., 2002). Because of the thin nature of this bed, most well logs pick the base of the Hell Creek Formation at the top of a 5- to 15-m-thick clean sandstone, representing the uppermost Colgate Member of the Fox Hills Formation (Murphy et al., 2002).

Secularists have tried to date the base of the Hell Creek Formation using argon-argon dates from volcanic ash along the North Dakota-South Dakota boundary but have not obtained a tight cluster, finding a range of values that varied as much as 1.32 my (Hicks et al., 2002), almost the entire 1.36-my interval claimed for deposition of the complete Hell Creek Formation (Hicks et al., 2002). Because of these age discrepancies, the base of the unit is thought to be significantly diachronous (Johnson et al., 2002). The base of the Hell Creek Formation is thought to be older in western North Dakota and progressively younger to the east (Johnson and Hickey, 1990).

Top of the Formation

The top of the Hell Creek Formation also can be difficult to identify in the field (Nichols and Johnson, 2002). Most geologists pick it at the base of a lignite or coal bed in the overlying Ludlow Member of the Fort Union Formation called the "Z" coal (Murphy et al., 2002; see Figure 2). However, this lignite bed is not always present (Nichols and Johnson, 2002). Where absent, the upper limit of the Hell Creek Formation is chosen where the sediments change from a smectite-rich, "popcorn"-weathering, gray mudstone to a carbonaceous shale, yellowish mudstone, or yellowish sandstone (Nichols and Johnson, 2002).

The contact between the Fort Union and the Hell Creek formations is believed to be nearly isochronous over more than 500 km, in contrast to the base of the formation (Johnson et al., 2002). According to Johnson et al. (2002), this implies a rapid transgression of the Paleocene seaway across the Hell Creek Formation, which deposited the Cannonball Member of the Fort Union Formation. This likely marks the onset of the Tejas Megasequence across western North America.

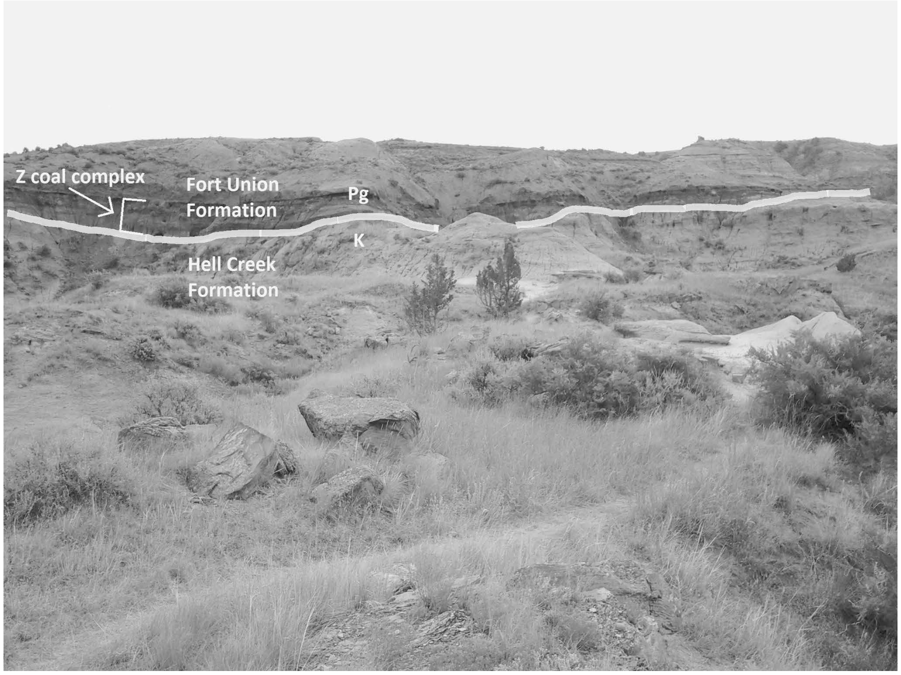


Figure 2. Photograph of the boundary between the Hell Creek Formation and the overlying Fort Union Formation near Glendive, Montana, showing the "Z coal complex" at the base of the Fort Union (Tullock Member). Photo courtesy of Brian Thomas.

The age of the top of the unit is, however, poorly constrained. Geologists admit that the top of the Hell Creek Formation does not necessarily coincide with the K-Pg (K-T) boundary (Johnson, 1992; Murphy et al., 2002). The K-Pg has been found to be as much as 2.6 m above the top of the Hell Creek Formation (Murphy et al., 2002). The K-Pg boundary used to be designated directly above the uppermost occurrence of dinosaur bones, but that idea has fallen from favor (Clemens and Hartman, 2014). Geologists now rely primarily on the Ir anomaly found in many marine sections throughout the world (Alvarez et al., 1980). But the presence of an Ir anomaly is not ubiquitous across the top of the Hell Creek Formation, as discussed below.

The presence of an Ir anomaly was found in samples from the Z coal (and above) at two sites in Garfield County, Montana, between 1980 and 1982, one near Brownie Butte in the Hell Creek valley, and the other at a location called Ir Hill on the southwestern margin of Hauso Flats (Clemens and Hartman, 2014). This iridium-rich layer has been labeled the IrZ. The sample from near Brownie Butte was collected from a thin clay layer identified by palynology as the K-Pg boundary. However, subsequent samples taken from Brownie Butte and adjacent hills did not contain the same IrZ layer and did not have high levels of Ir at the base of the Z coal (Clemens and Hartman, 2014).

Ash taken from the IrZ layer in the valley of Hell Creek was dated by geologists, using ^{40}Ar/^{39}Ar, giving an age of 65.58 ± 0.04 ma (Wilson, 2005). To the east in McCone County, however, the same basal Z coal, ash-rich layer gave an age of 65.37 ± 0.05 ma (Clemens and Hartman, 2014). Secularists are quick to claim that these are close but admit the differences in dates make it very difficult to explain the top of the Hell Creek Formation as an isochronous surface (Clemens and Hartman, 2014). Although secular geologists would like to see a universal Ir anomaly across the top of the Hell Creek Formation, they do not. They admit that the evidence for an asteroid impact, like the claimed Chicxulub site, is lacking within most of the exposures of the uppermost Hell Creek Formation, finding the IrZ anomaly in only a few isolated sections (Clemens and Hartman, 2014).

It appears that the only reason the K-Pg and the top of the Hell Creek Formation do not always coincide is the sporadic Ir anomaly. When it is present, secular geologists set the K-Pg boundary to match it, not the lithologic formation top. Sometimes, however, they adjust the top of the Hell Creek to match the Ir anomaly, as in Figure 3. The physical top of the Hell Creek should be placed a few meters higher than the Ir anomaly, at the top of their unit 29, in the lectostratotype section (Figure 3). This matches a clearer change in rock type, which normally defines a formational contact. Unfortunately, secular scientists insist on using the Ir anomaly, in spite of its unmappable nature, and choose to ignore the obvious stratigraphic conflict it sometimes creates.

Stratigraphy of the Formation

Detailed stratigraphic studies of the Hell Creek Formation include those by Belt et al. (1997), Butler (1980), Fastovsky (1986), Johnson (1989, 1992), and Murphy et al. (2002). Most of these studies include descriptions of measured sections and formation contact descriptions. Belt et al. (1997) and Fastovsky (1986) found paleocurrent directions in the Hell Creek in Montana and North Dakota to be predominantly to the south-southeast.

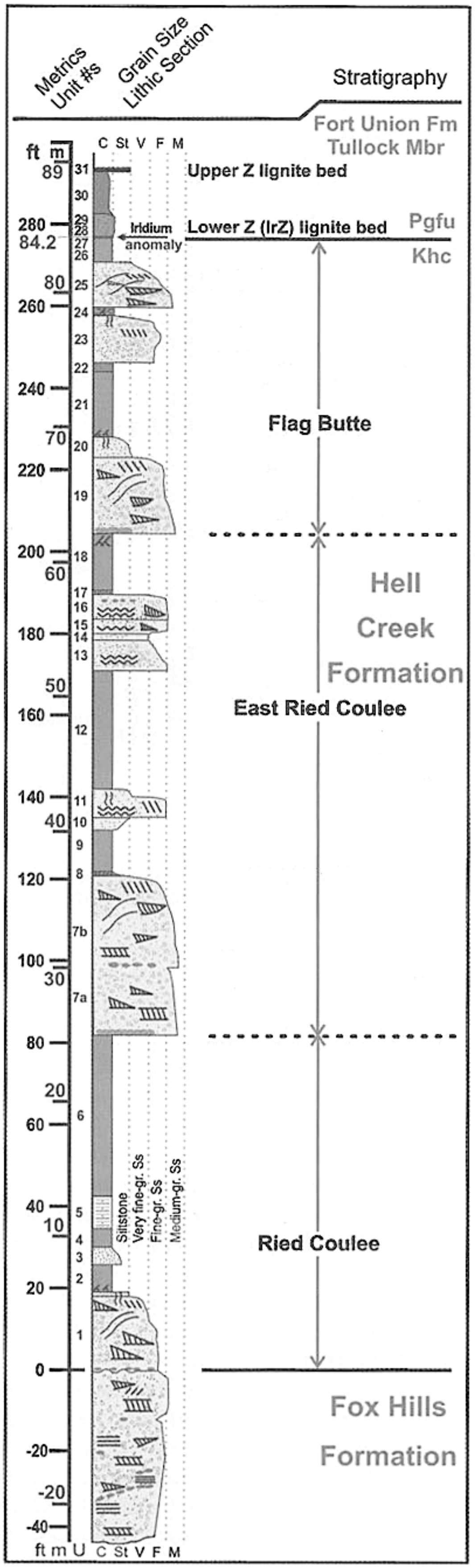
The most recent stratigraphic study was by Hartman et al. (2014), proposing a "type section" for the Hell Creek Formation (Figure 3). They noted that when he named the formation, Brown (1907) did not provide such a top-to-base reference section, as is the convention in modern stratigraphy.

The proposed type section, termed a lectostratotype location, described by Hartman et al. (2014), is located in the Flagg Butte area, just north of Jordan, Montana, near exposures along Ried Coulee in Garfield County. This location is about 26.7 km east of Ir Hill, described above. The type section is actually a composite of two closely spaced locations in the N½ Sec. 29, T. 21 N., R. 38 W. At this location, the Hell Creek Formation was determined to be 84.2 m thick.

Moore et al. (2014) identified an Ir anomaly just below the top of the Hell Creek Formation in a thin pinkish claystone layer near the lowest thin coal bed at the nearby Flagg Butte. Hartman et al. (2014) noted that most of the 4.5-m-thick section above the Ir anomaly layer looked more like Fort Union sediments than Hell Creek sediments. Regardless, the K-Pg boundary picked in their type section was chosen on the basis of the Ir anomaly and not on the observed changes on sedimentology.

The upper contact at this location is the base of the IrZ coal bed, directly above an Ir anomaly. Hartman et al. (2014) further divided the type section into three informal units from bottom to top, the Ried Coulee, East Ried Coulee, and Flag Butte units, respectively. The Hell Creek Formation is essentially a repeating series of sandstone layers separated by mudstones. The three subdivisions of the formation are all interpreted by secularists as representing various types of coastal-deltaic environments that thin to the east and northeast in the Williston Basin. However, they do acknowledge that the sea level fluctuated and the Western Interior Sea had a strong influence on the deposition of the Hell Creek (Hartman et al., 2014).

Every sandstone unit within the Hell Creek Formation shows evidence of water transport and deposition (Hartman et al., 2014). The lectostratotype in Figure 3 has multiple examples of planar and wedge cross-bedded sandstones,



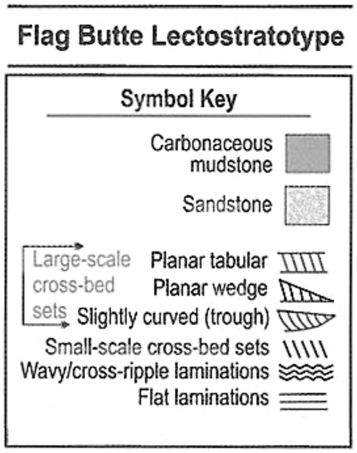

Flag Butte Lectostratotype
Symbol Key
Carbonaceous mudstone
Sandstone
Large-scale cross-bed sets
Planar tabular
Planar wedge
Slightly curved (trough)
Small-scale cross-bed sets
Wavy/cross-ripple laminations
Flat laminations

Figure 3 *(left)*. Type section of the Hell Creek Formation (lectostratotype) at Flagg Butte, located north of Jordan, Montana, in Garfield County. Thickness numbers start at the base of the formation. Grain size values: C—clay, St—silt, V—very fine-grained sand, F—fine-grained sand, and M—medium-grained sand. Ripples and cross-bedded sandstones indicative of water transport are found in every sandstone layer. Modified from Hartman et al. (2014).

rippled sandstones, and occasional sandstones with layers of rip-up clasts from higher energy flow. Most secular scientists interpret these sandstone layers as river channels, delta channels and/or overbank deposits (Hartman et al., 2014). However, the presence of marine fossils throughout the Hell Creek (see below discussion) strongly suggests that these sandstone layers were not terrestrial channels but were deposited under a strong marine influence. These sandstones and fossils, and therefore the entire Hell Creek, are best interpreted as forming under Flood conditions, where waves of ocean water washed across the continent, mixing land and sea creatures in water-deposited sands and mudstones.

Breien Member: Evidence of Marine Mixing

Although claimed to be a terrestrial deposit by the secular geologists, the Breien Member of the Hell Creek Formation contains ample evidence of marine influences and mixing throughout (Archibald, 1996; Hartman and Kirkland, 2002).

The Fox Hills Formation directly below the Hell Creek is interpreted as a marine deposit (Hartman, 2002), and the Cannonball Member of the Fort Union Formation above the Hell Creek is thought by secular geologists to be a marine deposit along the eastern side of the Williston Basin (Figure 3). Finally, most geologists admit that the Breien Member, near the base of the Hell Creek in south-central North Dakota, is a marine deposit. This unit covers an area of over 6100 km^2 (Murphy et al., 2002). Frye (1969) noted that the Breien Member is characterized by glauconitic sandstones that contain the marine crustacean trace fossil, *Ophiomorpha major*. He further added that the Breien Member contains marine, brackish water, freshwater, and land fossils. These are just the types of mixed fossils creationists would predict for a Flood deposit. Murphy et al. (2002) described the unit

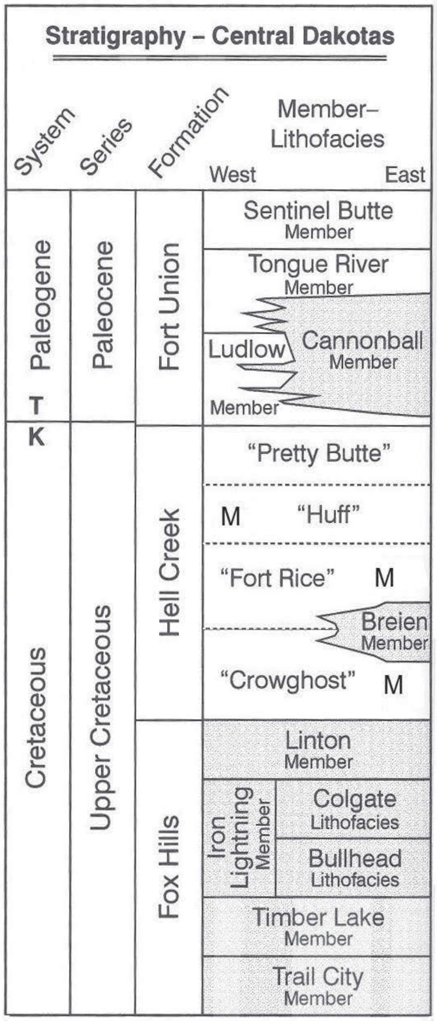


Figure 4. Stratigraphic units in the western and eastern Williston Basin of the Dakotas, showing the formations and members above and below the Hell Creek Formation. The Tullock Member (not shown) is the Ludlow Member equivalent in Montana. The shaded units are secular-accepted marine deposits. The white units are purported to be terrestrial deposits. The stippled boundaries in the Hell Creek Formation represent informal members named by Frye (1969) in North Dakota. The "M" designates layers with fossils providing a positive identification of marine taxon (Hartman and Kirkland, 2002). Modified from Hartman (2002) and Hartman and Kirkland (2002).

as rich in sandstone, mudstone, and occasional thin bentonite layers between 1.5 and 9 m thick near the base of the Hell Creek (Figure 4).

Murphy et al. (2002) also noted that 95% of the dinosaur fossils found throughout the Hell Creek Formation in south-central North Dakota, occur in close association with the Breien Member (within 10 m above or below).

In addition, Murphy et al. (2002) noted marine and/or brackish water fossils in the Cantapeta Tongue near the top of the Hell Creek Formation in south-central North Dakota. They concluded that marine or brackish conditions existed in southwestern North Dakota during much of the deposition of the Hell Creek. Note that the type section in Figure 3 is too far west to identify the Cantapeta Tongue and the Breien Member within the Hell Creek.

Fossil Occurrences and Marine Mixing

Hartman and Kirkland (2002) reported that occurrences of marine invertebrate fossils are more common throughout the Hell Creek Formation than most geologists would readily acknowledge. They argue that an ocean persisted across the Great Plains region throughout the end of the Cretaceous system with, at most, a brief hiatus at the K-Pg boundary. They concluded that the Western Interior Seaway never significantly regressed during deposition of the Hell Creek and that a nonmarine K-Pg interval did not occur in the central interior of North America.

Hartman and Kirkland (2002; see their Figure 3) used the four informal divisions of the Hell Creek Formation of Frye (1967, 1969) and noted the occurrence of marine fossils in three of the four. They also observed the presence of brackish water and marine bivalves *Crassostrea*, *Corbicula*, and gastropod *Pachymelania*, and the crustacean trace fossil *Ophiomorpha* throughout the Hell Creek. They also documented the presence of freshwater mollusks intermixed with the marine fossils in several of their subdivisions. As a conclusion, they suggestion that "the presence of marine waters very near the end of the Cretaceous seems a likely possibility" (Hartman and Kirkland, 2002, p. 272). They further concluded that the rock record in North Dakota does not support a withdrawal of marine conditions in the Western Interior prior to the end of the Cretaceous.

However, the marine influence seen in the Hell Creek Formation is not limited just to North Dakota. Studies in eastern Montana, and even into the equivalent rock units (Lance Formation) in Wyoming, have shown similar results (Archibald, 1996). Other authors have been studying the fauna of the Hell Creek since the 1950s (Lucas, 2007), seeing the same marine and nonmarine faunal mixing. As Hartman and Kirkland (2002, p. 272) have stated, "Although previously reported, knowledge of the continuation of marine conditions above the Fox Hills Formation is not well or widely known." The Fox Hills directly underlies the Hell Creek (Figure 3).

Hartman and Kirkland (2002, p. 276) also discussed the Hell Creek Formation research conducted by C. I Frye (1967, 1969). They pointed out that "Frye's identifications of brackish-water molluscan taxa, although in need of revision, were sufficient to deduce marine-influenced deposition through most of the Hell Creek Formation."

Table 2 shows a sampling of 12 groups of animals from the Hell Creek Formation and the overlying Tertiary (Paleogene) Tullock Member (basal unit of the Fort Union Formation; Figure 4) in Montana (Lucas, 2007). This table is the compilation of work begun in the 1950s by William Clemens and Robert Sloan and their colleagues (Lucas, 2007). The table data was supplemented with data from Archibald (1996), who reported similar findings in eastern Montana. Archibald's database included

Animal Group	Cretaceous Species Present	Paleogene Species Present
Sharks	5	0
Bony Fishes	14	7
Amphibians	8	6
Champosaurs	1	1
Crocodiles	5	3
Bird-hipped Dinosaurs	11	0
Lizard-hipped Dinosaurs	9	0
Lizards	11	3
Turtles	19	14
Placental Mammals	6	6
Marsupials	11	1
Multituberculates	10	5

Table 2. List of animal groups found as fossils in the Hell Creek Formation and in the overlying Tullock Member of the Fort Union Formation, eastern Montana. Note the mixing of marine and terrestrial species in the Hell Creek Formation and in the overlying Tullock Member. This mixing is best explained by Floodwaters transporting ocean animals onto the continent.

more than 150,000 vertebrate specimens housed in the University of California, Berkeley collection from Garfield and McCone counties, Montana.

Table 2 illustrates the many examples of mixed terrestrial and marine influences in the upper Hell Creek Formation in Montana, reinforcing the results of Hartman and Kirkland (2002) in North Dakota. Surprisingly, in two volumes of papers published specifically on the Hell Creek in the last 15 years (Geological Society of America Special Paper 361, 2002, and Special Paper 503, 2014), little is mentioned of the occurrences of the 5 species of sharks and the 14 species of fish that provide evidence of marine influence. Secular scientists either ignore these findings (e.g., Hartman and Kirkland, 2002, pp. 289–293) or dismiss them as freshwater species, in spite of the more likely conclusion that they represent marine organisms.

Formations directly under and over the Hell Creek Formation are accepted as marine, yet the Hell Creek itself is supposed to be terrestrial because it contains dinosaur fossils (Figure 4). Furthermore, the formation is also full of marine fossils—from top to bottom and from Montana to North Dakota. These facts are not best explained by fluctuating sea levels as most secularists claim. Frye (1969, p. 36) recognized that transport was necessary to mix these environments, if even only locally, stating, "These [marine] fossils were found at nearly every location and would lead one to believe that probably all of the terrestrial and most of the fresh-water fossils were washed into a marine or brackish-water environment." An event of tremendous energy was necessary to transport and mix marine and terrestrial animals we observe in the rocks today. The Flood could have provided this energy with its tsunami-like waves crashing across the continent.

The "3 Meter Gap"

Countless studies have argued about the extinction of dinosaurs in the secular literature, and the Hell Creek Formation continues to be at the forefront of this debate. Lucas (2007) reported that there was a steplike decline in the dinosaur diversity near the top of the Hell Creek Formation, with 19 genera near the base, declining to 12 within the top 16 m, and just 7 approximately 3 m from the top. But there have been no genera identified in the uppermost 3 m of the formation. Most of these data came from studies by Robert Sloan and William Clemens, mentioned earlier. Archibald (1998) reported similar findings, citing it as evidence for a "gradual" demise of the dinosaurs. Others disagree, insisting that there was no decline in dinosaur diversity and that dinosaur extinction was the result of a sudden, asteroid-driven extinction event (Fastovsky and Weishampel, 1996; Sheehan et al., 2000).

David Archibald (2000, p. 1150) has written, "A far more important question is the pattern and amount of extinction of all vertebrates, not just dinosaurs." Robert Bakker likes to look at what did not become extinct across the K-Pg boundary. He points out that frogs and turtles showed little, if any, change in population from the Cretaceous system to the Tertiary system (History Channel, 2008; cf. Paleogene, Table 2). He argues that global wildfires, acid rain, toxic dust, and extended global cooling after a large asteroid impact should have wreaked havoc on nearly all living things, especially those in the tropics, like frogs and turtles. As Bakker (History Channel, 2008) has stated, "If you can't freeze a turtle, you can't freeze a *T. rex*. You just can't." Research also has found little change in insect populations across the K-Pg boundary, further adding to the mystery of what did not go extinct (History Channel, 2008).

Finally, there is the near void of dinosaur skeletal remains in the last 3 m of the Cretaceous; the infamous "3 m gap." Critics of the asteroid hypothesis argue that there should be some skeletal evidence of a mass kill in rocks, like the uppermost Hell Creek Formation, if billions of animals across the globe died simultaneously. All that has been found in this 3-m interval are a few scattered dinosaur bones, with no articulated skeletons at all. Because of these isolated fossil occurrences, Clemens (2002) argued that the "gap" is really only the uppermost 2 m of the Hell Creek Formation. But, he points out that after 30 years of intense study, the 2-m gap remains. And it is not just dinosaurs missing in this 2-m zone; it is all vertebrates and even the "freshwater" invertebrates (Clemens, 2002). So whether it is a 2-m or a 3-m gap, it remains a mystery to the secular geologists. They cannot seem to square it with their asteroid theory.

Dinosaur Bones Found above the K-Pg

Sloan et al. (1986) published evidence of dinosaur fossils found above the Hell Creek Formation in Paleocene series rocks. These results also support the interpretation that dinosaur numbers were declining by the end of Hell Creek deposition. Based on the lack of articulated skeletons found in Paleocene rocks, Lofgren et al. (1990) had previously interpreted these "Paleocene" dinosaur fossils as reworked material, where later erosional events dug up the bones and redeposited them in younger sediments. In contrast, Argast et al. (1987) did experimental studies on fossil reptile and dinosaur teeth and found that very little abrasion occurs with transport (reworking). They concluded that it is impossible to tell whether or not these Paleocene fossils were transported.

The presence of dinosaur bones in rocks above the K-Pg boundary is seen in other formations. Dinosaur fossils in sediments above the K-Pg, mostly isolated teeth and bones, but some egg shells, have been found in many locations across the globe—in the United States, China, Bolivia, India, and Peru (Lucas, 2007). It appears the K-Pg was not a "magic" line in the rock record where dinosaurs suddenly disappeared. Clarey and Heim (2012) came to a similar conclusion in their study of K-Pg extinctions of all types of organisms across the world. Landman et al. (2014) also reported that selected ammonite species were found in lower Paleocene series sediments, demonstrating that they did not all disappear at the K-Pg either.

Zuni Megasequence

Megasequence boundaries appear to be very important in understanding both the history of the Flood and secularist paleontologists' perception of extinctions (Snelling, 2014a). The Flood seems to have buried most of the dinosaurs below the level of the K-Pg, but the Zuni Megasequence extends a bit higher, into lowermost Paleocene series deposits (Blakey, 2010). Unfortunately, a "transposing" error shows the top of the Zuni Megasequence too high stratigraphically in Snelling (2014a) and Morris (2012). It seems likely that the top of the Zuni Megasequence, not the K-Pg, marks the true level of the disappearance of dinosaurs in Flood strata. This interpretation also fits better with assumed tsunami-like waves envisioned for the Flood deposits and for the megasequences.

For Flood geology, the relationship of air-breathing terrestrial fauna, the biblical record of their death within the first 150 days of the Flood, and their stratigraphic location in the geologic column has been of considerable interest (Snelling, 2014a). If the Hell Creek Formation represents a later time during the Flood, then we must explain why dinosaur fossils are found in it. It is possible that dinosaurs were able to survive up to this point in the Flood due to the lower volume of sediment accumulation in the first three megasequences, compared to the later megasequences. In fact, preliminary calculations, based on my unpublished 3-D model of sediment volume across the USA, show only about one-third of the Flood sediments were deposited in the early stages of the Flood (the first three megasequences), and most of this was east of the modern Mississippi River. It was the latter part of the still rising Flood, when the sedimentation volume greatly increased (the last three megasequences), especially in the West, that the dinosaurs were trapped in mud and sand and buried.

Dinosaurs Buried in the Later Flood Strata

There are problems with all proposed Flood models, especially in reconciling the timing of biblical events with the stratigraphic location of fossil evidence. Everything from ecological zonation to the rejection of the chronostratigraphic timescale has been proposed, but no solution has been universally accepted (Coffin, 1983; Froede and Akridge, 2013; Ross, 2013; Snelling, 2014a; Walker, 1994). Catastrophic plate tectonics (CPT) has been proposed as an explanation for the observable geology (Austin et al., 1994; Snelling, 2014b) and as an explanation for the megasequences (Snelling, 2014a). CPT is the working model used for the following discussion and interpretations.

After the early stages of the Flood, and after the deposition of the first three megasequences (Sauk, Tippecanoe, and Kaskaskia), the Flood seems to have increased its fury and energy level, depositing nearly two-thirds of its sediment load onto the North American continent. "And the waters prevailed, and were increased greatly upon the earth; and the ark went upon the face of the waters. And the waters prevailed exceedingly upon the earth; and all the high hills, that were under the whole heaven, were covered" (Genesis 7:18–19 KJV).

It was at this point in the Flood, during deposition of the Absaroka and Zuni Megasequences, that the tectonic plates seem to have undergone their most extensive episode of movement and an entirely new ocean crust began to form globally. The new ocean crust would likely have been hotter and less dense than the old, pre-Flood oceanic crust. This would have raised the level of the ocean floor, especially near the ocean ridges, similar to the way hot air causes a balloon to rise (Austin et al., 1994). Some creation scientists have estimated this action alone could have raised global sea level by as much as 1.6 km, greatly helping to flood the land masses (Snelling, 2014b).

Subduction commenced along the west coast of North America (and other locations around the Pacific) during the deposition of the Absaroka Megasequence, pulling down part of the crust of North America along with it (Baumgardner, 2005). This would have created accommodation space for tremendous volumes of sediment on the former land surface. Huge tsunami-like sand waves likely traversed from west to east across much of the continent. Evidence of these large sand waves is found in the thick, cross-bedded sandstones of the Permian and Jurassic system rocks (part of the Absaroka Megasequence) in the western United States.

In this scenario, the dinosaurs would have been quickly inundated by the accumulating wedge of thousands of meters of sediment rapidly deposited across many Western states. Due to elevation differences (see Dinosaur Peninsula in Clarey, 2015), this sediment incursion would have been initially less intense to the north. Thus, the remaining land refuges of the dinosaurs were systematically buried from south to north. This violent event would have also transported large marine vertebrates onto the continent, depositing them in the same sediments as the dinosaurs, and resulting in the intermixing of marine, terrestrial and freshwater fauna found in the Hell Creek Formation.

Post-Flood erosion, including the effects of the Ice Age (Oard, 1990), has removed any later Flood strata (Tejas Megasequence) that were likely deposited on top of the Hell Creek, leaving it exposed at the surface today.

Discussion and Conclusions

The Hell Creek Formation of Montana, North Dakota, and South Dakota (Figure 1) is one of the most intensely studied sedimentary units in North America. It is used, and possibly overused, in the K-Pg extinction debate over why dinosaurs suddenly disappear in the rock record near the end of the Cretaceous system. The nature of the dinosaur extinction remains one of the biggest mysteries in paleontology for secular scientists. It is likewise a mystery for creationists trying to reconcile the timing of Flood events, including the death of the dinosaurs (those not on the ark) in the first 150 days of the Flood, their stratigraphic position, and their post-Flood extinction.

Disagreement over stratigraphic techniques has caused additional problems. Since the advent of the impact hypothesis, scientists have made the stratigraphic location of the Ir anomaly their primary criterion for picking the K-Pg boundary. However, that anomaly is not geographically continuous; it exists only in some locations near the top of the Hell Creek Formation. This reliance on the Ir anomaly has made picking the top of the Hell Creek Formation nebulous. Instead of the traditional criterion of a mappable change in lithology, many geologists try to map the Ir anomaly. In addition to the increased geographic uncertainty, its location a few meters away from the traditional boundary has created differences in the formation top and the K-Pg boundary of several meters, adding to the confusion.

Two points strongly suggest the secular depositional model for the Hell Creek Formation is in error. First, many marine fossils are found throughout the formation, often mixed with terrestrial fossils. The extent of these occurrences has been largely ignored by secular scientists. Second, sedimentary structures indicative of water transport are ubiquitous to the sandstone layers. Rippled layers and cross-bedded sandstones demonstrate active transport was occurring during deposition of each sandstone bed. Standard models cannot account for both the mixed fossil assemblages and the evidence of water transport. Only a Flood model involving ocean-transported, tsunami-like waves can account for the observed mixing of terrestrial and marine environments in the Hell Creek.

In a Flood model, the debate over a sudden or a gradual extinction to the dinosaurs is immaterial. There are no true extinctions as popular literature likes to describe. “Extinctions” are merely the last occurrence of a particular organism in Flood sediments. The Hell Creek Formation does seem to contain fewer and less diverse dinosaur fossils toward the top, and there is the 2–3-m “gap” at the very top with no dinosaur fossils. However, this does not mean dinosaurs were slowly or rapidly dying out as the Hell Creek was being deposited. It shows the general point in the rock record where the last of the pre-Flood dinosaurs succumbed to the rising Floodwaters. And the isolated discoveries of post-Hell Creek dinosaur fossils may suggest that a few dinosaurs were able to survive a bit longer than even the top of the Hell Creek, becoming buried in the lowermost Paleocene Series sediments. This slightly higher stratigraphic level coincides with the end of the Zuni Megasequence, suggesting this event may be more significant in a Flood model than the top of the Hell Creek Formation.

Acknowledgments

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References

Alvarez, L.W., W. Alvarez, F. Asaro, and H.V. Michel. 1980. Extraterrestrial cause for the Cretaceous-Tertiary extinction. *Science* 208:1095–1108.

Archibald, J.D. 1996. *Dinosaur Extinction and the End of an Era*. Columbia University Press, New York, NY.

Archibald, J.D. 1998. Death, taxes, and extinction. *Phi Kappa Phi Journal* 78(3): 32–35.

Archibald, J.D. 2000. Dinosaur abundance was not declining in a "3 m gap" at the top of the Hell Creek Formation, Montana and North Dakota. Comment. *Geology* 28(12): 1150.

Argast, S., J.O. Farlow, R.M. Gabet, and D.L. Brinkman. 1987. Transport-induced abrasion of fossil reptilian teeth: implications for the existence of Tertiary dinosaurs in the Hell Creek Formation, Montana. *Geology* 15:927–930.

Austin, S.A., J. R. Baumgardner, D.R. Humphreys, A.A. Snelling, L. Vardiman, and K.P. Wise. 1994. Catastrophic plate tectonics: a global Flood model of earth history. In Walsh, R.E. (editor), *Proceedings of the Third International Conference on Creationism*, pp. 609–622. Creation Science Fellowship, Pittsburgh, PA.

Bauer, C.M. 1925. Quartzite pebbles at the base of the Lance Formation in Montana. *American Association of Petroleum Geologists Bulletin* 9:344–346.

Baumgardner, J.R. 2005. Recent rapid uplift of today's mountains. *Acts & Facts* 34(3).

Belt, E.S., J.F. Hicks, and D.A. Murphy. 1997. A pre-Lancian regional unconformity and its relationship to Hell Creek paleogeography in southeastern Montana. *Contributions to Geology* 31(2): 1–26.

Benton, M.J. 1990. Scientific methodologies in collision: the history of the study of the extinction of dinosaurs. *Evolutionary Biology* 24:371–400.

Blakey, R. 2010. Paleogeographic and paleotectonic maps—use, construction, interpretation, and example. Rocky Mountain Association of Geologists Short Course, January 28, 2010, Denver, CO.

Bohor, F.B., E.E. Foord, P.J. Modreski, and D.M. Triplehorn. 1984. Mineralogic evidence for an impact event at the Cretaceous-Tertiary boundary. *Science* 224:867–869.

Brown, B. 1907. The Hell Creek beds of the Upper Cretaceous of Montana. *American Museum of Natural History Bulletin* 23:823–845.

Butler, R.D. 1980. Stratigraphy, sedimentology, and depositional environments of the Hell Creek Formation (Late Cretaceous) and adjacent strata, Glendive area, Montana. Ph.D. diss., University of North Dakota, Grand Forks, ND.

Carlisle, D.B. 1992. Diamonds at the K/T boundary. *Nature* 357:119–120.

Clarey, T. 2015. Dinosaur fossils in late-Flood rocks. *Acts & Facts* 44(2).

Clarey, T.L, and K.A. Heim. 2012. A review of extinction patterns across the K-Pg boundary. *Journal of Creation Theology and Science Series C: Earth Sciences* 2:1–2.

Clemens, W.A. 2002. Evolution of the mammalian fauna across the Cretaceous-Tertiary boundary in northwestern Montana and other areas of the Western Interior. In Hartman, J.H., K.R. Johnson, and D.J. Nichols (editors), *The Hell Creek Formation and the Cretaceous-Tertiary Boundary in the Northern Great Plains: An Integrated Continental Record of the End of the Cretaceous*, pp. 217–245. Geological Society of America Special Paper 361, Boulder, CO.

Clemens, W.A., and J.H. Hartman. 2014. From *Tyrannosaurus rex* to asteroid impact: early studies (1901–1980) of the Hell Creek Formation in its type area. In Wilson, G.P., W.A. Clemens, J.R. Horner, and J.H. Hartman (editors), *Through the End of the Cretaceous in the Type Locality of the Hell Creek Formation in Montana and Adjacent Areas*, pp. 1–87. Geological Society of America Special Paper 503, Boulder, CO.

Coffin, H.G. 1983. *Origin by Design*. Review and Herald Publishing Association, Hagerstown, MD.

Fastovsky, D.E. 1986. Paleoenvironments of vertebrate-bearing strata at the Cretaceous-Paleogene boundary in northeastern Montana and southwestern North Dakota. Ph.D. diss., University of Wisconsin, Madison, WI.

Fastovsky, D.E., and D.B. Weishampel. 1996. *The Evolution and Extinction of the Dinosaurs*. Cambridge University Press, Cambridge, England.

Froede, C., and A.J. Akridge. 2013. A developing schism in Flood geology. *Journal of Creation* 27(2): 49–54.

Frye, C.I. 1967. The Hell Creek Formation in North Dakota. Ph.D. diss., University of North Dakota, Grand Forks, ND.

Frye, C.I. 1969. Stratigraphy of the Hell Creek Formation in North Dakota. *North Dakota Geological Survey Bulletin* 54:1–65.

Hartman, J.H. 2002. Hell Creek Formation and the early picking of the Cretaceous-Tertiary boundary in the Williston basin. In Hartman, J.H., K.R. Johnson, and D.J. Nichols (editors), *The Hell Creek Formation and the Cretaceous-Tertiary Boundary in the Northern Great Plains: An Integrated Continental Record of the End of the Cretaceous*, pp. 1–7. Geological Society of America Special Paper 361, Boulder, CO.

Hartman, J.H., and J.I. Kirkland. 2002. Brackish and marine mollusks of the Hell Creek Formation of North Dakota: evidence for a persisting Cretaceous seaway. In Hartman, J.H., K.R. Johnson, and D.J. Nichols (editors), *The Hell Creek Formation and the Cretaceous-Tertiary Boundary in the Northern Great Plains: An Integrated Continental Record of the End of the Cretaceous*, pp. 271–296. Geological Society of America Special Paper 361, Boulder, CO.

Hartman, J.H., R.D. Butler, M.W. Weiler, and K.K. Schumaker. 2014. Context, naming, and formal designation of the Cretaceous Hell Creek Formation lectostratotype, Garfield County, Montana. In Wilson, G.P., W.A. Clemens, J.R. Horner, and J.H. Hartman (editors), *Through the End of the Cretaceous in the Type Locality of the Hell Creek Formation in Montana and Adjacent Areas*, pp. 89–121. Geological Society of America Special Paper 503, Boulder, CO.

Hicks, J.F., K.R. Johnson, J.D. Obradovich, L. Tauxe, and D. Clark. 2002. Magnetostratigraphy and geochronology of the Hell Creek and basal Fort Union Formations of southwestern North Dakota and a recalibration of the age of the Cretaceous-Tertiary boundary. In Hartman, J.H., K.R. Johnson, and D.J. Nichols (editors), *The Hell Creek Formation and the Cretaceous-Tertiary Boundary in the Northern Great Plains: An Integrated Continental Record of the End of the Cretaceous*, pp. 35–55. Geological Society of America Special Paper 361, Boulder, CO.

Hildebrand, A.R., G.T. Penfield, D.A. Kring, M. Pilkington, Z.A. Camargo, S.B. Jacobsen, and W.V. Boynton. 1991. Chicxulub crater: a possible Cretaceous-Tertiary boundary impact site on the Yucatan Peninsula, Mexico. *Geology* 19:867–871.

History Channel. 2008. *The First Apocalypse*. https://www.youtube.com/watch?v=g7tg7O48bJU.

Jensen, F.S., and Varnes, H.D. 1964. Geology of the Fort Peck Area, Garfield, McCone, and Valley Counties, Montana. *U.S. Geological Survey Professional Paper 414F*.

Johnson, K.R. 1989. A high resolution megafloral biostratigraphy spanning the Cretaceous-Tertiary boundary in the Northern Great Plains. Ph.D diss., Yale University, New Haven, CT.

Johnson, K.R. 1992. Leaf-fossil evidence for extensive floral extinction at the Cretaceous-Tertiary boundary, North Dakota, USA. *Cretaceous Research* 13:91–117.

Johnson, K.R., and L.J. Hickey. 1990. Megafloral change across the Cretaceous/Tertiary boundary in the northern Great Plains and Rocky Mountains, U.S.A. In Sharpton, V.L., and P.D. Ward (editors), *Global Catastrophes in Earth History: An Interdisciplinary Conference on Impacts, Volcanism, and Mass Mortality*, pp. 433–444. Geological Society of America Special Paper 247, Boulder, CO.

Johnson, K.R., D.J. Nichols, and J.H. Hartman. 2002. Hell Creek Formation: A 2001 synthesis. In Hartman, J.H., K.R. Johnson, and D.J. Nichols (editors), *The Hell Creek Formation and the Cretaceous-Tertiary Boundary in the Northern Great Plains: An Integrated Continental Record of the End of the Cretaceous*, pp. 503–510. Geological Society of America Special Paper 361, Boulder, CO.

Keller, G., and T. Adatte. 2007. Age and paleoenvironment of Deccan volcanism and the K-T mass extinction. *Geological Society of America Annual Meeting Abstracts with Programs* 39(6): 566.

Keller, G., T. Adatte, Z. Berner, M. Harting, G. Baum, M. Prauss, A. Tantawy, and D. Stueben. 2007. Chicxulub impact predates K-T boundary: new evidence from Brazos, Texas. *Earth and Planetary Science Letters* 255:339–356.

Kuiper, K.F., A. Deino, F.J. Hilgen, W. Krijgsman, P.R. Renne, and J.R. Wijbrans. 2008. Synchronizing rock clocks of Earth history. *Science* 320:500–504.

Landman, N.H., S. Goolaerts, J.W.M. Jagt, E.A. Jagt-Yazykova, M. Machalski, and M.M. Yacobucci. 2014. Ammonite extinction and nautilid survival at the end of the Cretaceous. *Geology* 42(8): 707–710.

Lofgren, D.L., C.L. Hotton, and A.C. Runkel. 1990. Reworking of Cretaceous dinosaurs into Paleocene channel deposits, upper Hell Creek Formation, Montana. *Geology* 18:874–877.

Lucas, Spencer G. 2007. *Dinosaurs: The Textbook*. 5th edition. McGraw-Hill Higher Education, Boston, MA.

Morris, J.D. 2012. *The Global Flood: Unlocking Earth's Geologic History*. Institute for Creation Research, Dallas, TX.

Moore, J. R., G.P. Wilson, M. Sharma, H.R. Hallock, D.R. Braman, and P.R. Renne. 2014. Assessing the relationship of the Hell Creek-Fort Union contact, Cretaceous-Paleogene boundary, and Chicxulub impact ejecta horizon at the Hell Creek Formation lectostratotype, Montana, USA. In Wilson, G.P., W.A. Clemens, J.R. Horner, and J.H. Hartman (editors), *Through the End of the Cretaceous in the Type Locality of the Hell Creek Formation in Montana and Adjacent Areas*, pp. 123–135. Geological Society of America Special Paper 503, Boulder, CO.

Murphy E.C., J.W. Hoganson, and K.R. Johnson. 2002. Lithostratigraphy of the Hell Creek Formation in North Dakota. In Hartman, J.H., K.R. Johnson, and D.J. Nichols (editors), *The Hell Creek Formation and the Cretaceous-Tertiary Boundary in the Northern Great Plains: An Integrated Continental Record of the End of the Cretaceous*, pp. 9–34. Geological Society of America Special Paper 361, Boulder, CO.

Nichols, D.J., and K.R. Johnson. 2002. Palynology and microstratigraphy of Cretaceous-Tertiary boundary sections in southwestern North Dakota. In Hartman, J.H., K.R. Johnson, and D.J. Nichols (editors), *The Hell Creek Formation and the Cretaceous-Tertiary Boundary in the Northern Great Plains: An Integrated Continental Record of the End of the Cretaceous*, pp. 95–143. Geological Society of America Special Paper 361, Boulder, CO.

Oard, M.J. 1990. *An Ice Age Caused by the Genesis Flood*. Institute for Creation Research, El Cajon, CA.

Powell, J.L. 1998. *Night Comes to the Cretaceous*. W. H. Freeman and Company, New York, NY.

Ross, M.R. 2013. Letter: a developing schism in Flood geology. *Journal of Creation* 27(3): 46–47.

Sheehan, P.M., D.E. Fastovsky, C. Barreto, and R.G. Hoffmann. 2000. Dinosaur abundance was not declining in a "3 m gap" at the top of the Hell Creek Formation, Montana and North Dakota. *Geology* 28(6): 523–526.

Sloan, R.E., J.K. Rigby Jr., L. Van Valen, and D. Gabriel. 1986. Gradual dinosaur extinction and simultaneous ungulate radiation in the Hell Creek Formation. *Science* 232:629–633.

Sloss, L. 1963. Sequences in the cratonic interior of North America. *Geological Society of America Bulletin* 74:93–114.

Snelling, A.A. 2014a. Geological issues: charting a scheme for correlating the rock layers with the biblical record. In Boyd, S.W., and A.A. Snelling (editors), *Grappling with the Chronology of the Genesis Flood*, pp. 77–109. Master Books, Green Forest, AR.

Snelling, A.A. 2014b. Geophysical issues: understanding the origin of the continents, their rock layers and mountains. In Boyd, S.W., and A.A. Snelling (editors), *Grappling with the Chronology of the Genesis Flood*, pp. 111–143. Master Books, Green Forest, AR.

Thomas, B. 2015. Original biomaterials in fossils. *Creation Research Society Quarterly* 51:234–247.

Walker, T.B. 1994. A biblical geologic model. In Walsh, R.E. (editor), *Proceedings of the Third International Conference on Creationism*, pp. 581–592. Creation Science Fellowship, Pittsburgh, PA.

Wilson, G.P. 2005. Mammalian faunal dynamics during the last 1.8 million years of the Cretaceous in Garfield County, Montana. *Journal of Mammalian Evolution* 12:53–76.

Creation Research Society Quarterly 2015. 51:299–311.

Radiocarbon in Dinosaur and Other Fossils

Brian Thomas and Vance Nelson*

Abstract

Measurable amounts of radiocarbon have been consistently detected within carbonaceous materials across Phanerozoic strata. Under uniformitarian assumptions, these should no longer contain measurable amounts of radiocarbon. Secularists have asserted that these challenging finds originate from systematic contamination, but the hypothesis of endogenous radiocarbon should be considered. Assuming these strata were largely deposited by the Noahic Flood occurring within the time range of radiocarbon's detectability with modern equipment under uniformitarian assumptions, we hypothesized that fossils from all three erathems, including dinosaur fossils, should also contain measurable amounts of radiocarbon. Consistent with this hypothesis, we report detectable amounts of radiocarbon in all 16 of our samples. Attempts to falsify our hypothesis failed, including a comparison of our data with previously published carbon-dated fossils. We conclude that fossils and other carbonaceous materials found throughout Phanerozoic strata contain measurable amounts of radiocarbon that is most probably endogenous.

Introduction

The consistent failure of carbon dating facilities to find carbon-dead samples to serve as baselines highlights the regularity with which they have detected measurable amounts of radiocarbon in samples from Phanerozoic settings (Nadeau et al., 2001). Simply put, carbonaceous materials from any portion of the geologic column deposited millions of years ago should, with the exception of rare instances of contamination, contain zero ^{14}C atoms. This reasonable assumption follows from the half-life of ^{14}C, which places a time limit on its duration of about 100,000 years until the number of ^{14}C atoms become too few to detect with accelerator mass spectrometry (AMS) (Hebert, 2013). Preliminary results from undocumented dinosaur bones, where the bones were genuine but not curated into a repository, presented at the 1990 International Conference on Creationism (ICC) indicated measurable radiocarbon, but contamination could not be ruled out (Dahmer et al., 1990). At the 2003 ICC, the RATE team (a research effort sponsored by the Institute for Creation Research and the Creation Research Society and an acronym for "Radioisotopes and the Age of The Earth") argued that since Pennsylvanian coals used to gener-

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ate a baseline prior to dating a sample typically register amounts of radiocarbon well in excess of AMS detection limits, the radiocarbon must be endogenous to the coal, and not a contaminant (Baumgardner et al., 2003).

Consistent with this hypothesis, they found radiocarbon in ten carefully extracted coal samples and even in ten diamonds (Baumgardner, 2005). In addition, Snelling published a series of articles over a number of years describing individual carbon dates for fossils, including wood and shell samples (Snelling, 1998, 1999, 2000a, 2000b, 2008a, 2008b). These data confirm the expectation that endogenous radiocarbon might occur in dinosaur and other fossils. They also suggest a test of the Creation-Flood model. If it were possible to calibrate radiocarbon "ages" to calendar ages, date-specific amounts of ^{14}C within fossils could be compared to the expected age of Noah's Flood at 4300 or so years ago. However, until future studies establish such a calibration, a more generalized test presents itself. Namely, if Phanerozoic strata were not deposited hundreds or tens of millions of years ago, but by Noah's Flood fewer than 10,000 years ago, they should still contain detectable amounts of ^{14}C. We tested this possibility by obtaining and analyzing carbon dates for dinosaur bones, other fossil bones, and fossil wood.

Sample Preparation

A single lab used the following standard radiocarbon dating procedures in performing their AMS tests and calculating radiocarbon ages for all 16 of our samples. Preparation protocols for radiocarbon isotope analyses of bone apatite were performed according to Cherkinsky (2009). First, extraneous materials were removed by physical scraping. Then, samples were soaked overnight in 1N acetic acid. This removes carbon compounds that contaminate samples by sediment infilling or carbonate crystallization post-deposition. After rinsing and drying, approximately 2 grams of bone are crushed and retreated with 1N acetic acid with periodic evacuations until CO_2 and other gases cease forming. This acid treatment does not exceed 72 hours, after which time original bioapatite begins dissolving, not just secondary surface carbonaceous materials. After drying again, several hundred mg of partially treated bone are added to 1N HCl for fewer than 20 min, and CO_2 from the reaction is collected. If the mass of captured carbon exceeds expected amounts, contaminating contributions are suspected and additional acid treatments ensue. Finally, the cleaned carbon dioxide is catalytically converted to graphite for accelerator mass spectrometer analysis of the ^{13}C/^{14}C ratio that is immediately compared to the ^{13}C/^{14}C ratio in the absolute radiocarbon standard sample OXI (NBS 4990).

"Uncalibrated" radiocarbon dates in radiocarbon years "Before Present," defining "present" as AD 1950, a date that honors the publication of the first use of carbon date calculations (Arnold and Libby, 1949) are reported. These uncalibrated dates were calculated using the traditional, older, and less accurate Libby ^{14}C half-life of 5,568 years, and the error margins represent one standard deviation (1σ). This error expression incorporates both experimental and statistical errors. Further, the lab corrected each of our dates for isotope fractionation, which assumes an original ratio of stable ^{13}C. Although for the sake of completion it is important to disclose these standards, they have no bearing on the main findings of this present investigation. We are not attempting to use carbon dating to determine ages of artifacts but merely to test for the presence of measurable amounts of ^{14}C in fossils bearing conventional age assignments that should preclude ^{14}C. These radiocarbon results are summarized in Table 1 and described below.

Specimen Collection and Analysis

A Miocene Plant Specimen

We describe 14 recently carbon dated fossils that span all three erathems. Our *Tectocarya rhenana* specimen was obtained from the Braunkohle Lignite seam in Adendorf, Germany. The Alberta Dinosaur Museum (ADM) had procured a number of samples from secular geologic contacts from within the open fossil trading market, including this one. We could smell fruitlike odors emitting from these fossils. We submitted the fossil for chemical analysis, testing for the esters that confer fruity odors, and we plan to present those results elsewhere.

Our sample of the darkened fossil fruit was naturally mummified. We hypothesized, on the basis of the assumption that the lignite was deposited by a local catastrophe after Noah's Flood and was thus only several thousand years old, that it had not yet lost all of its radiocarbon atoms. According to the 2014 International Chronostratigraphic Chart, Middle Miocene fossils are assigned ages exceeding eleven million years. The sample was photographed and a portion packaged and mailed to a nationally recognized radiocarbon facility. We obtained 10.84 pMC for the fossil fruit, which corresponds to the age shown in Figure 1 of 17,850 ± 40 BP, where "BP" means "uncalibrated radiocarbon years Before Present." While the procedure for converting the uncalibrated years that radiocarbon dating facilities typically supply into calibrated years has been simplified using the free software OxCal, and whereas ^{14}C "ages" are most often calibrated to years BC, we saw no need to perform this step in the current analysis. The standard calibration procedure incorporates more uniformitarian assumptions than those already used in generating raw radiocarbon years and usually ends up adding a few thousand "years" to the number of reported raw radiocarbon years for ages as great as we

Taxon	Radio-carbon Years BP	pmc	δ13	Stratigraphy	Sample date	Note
Tectocarya rhenana	17850 ± 40	10.84	-25.4	Braunkohle Lignite	6/1/2011	mummified fruit
hadrosaur vert (ICR)	20850 ± 90	7.46	-24.51	Hell Creek Fm.	3/20/2013	Medullary bone
Edmontosaurus sp.	25550 ± 60	4.15	-0.5	Lance Fm.	5/30/2014	vertebra
Phareodus sp.	26,110 ± 60	3.87	-0.4	Green River Fm.	5/30/2014	skull bones & scales
ceratopsian	26300 ± 60	3.78	-3.6	Horseshoe Canyon Fm.	7/14/2014	metacarpal V
hadrosaur vert (ICR)	28790 ± 100	2.78	-20.11	Hell Creek Fm.	3/20/2013	cortical bone
Edmontosaurus sp.	32420 ± 160	1.77	-6.1	Lance Fm.	2/26/2013	phalanx
hadrosaur (ADM)	32770 ± 100	1.69	-3.5	Horseshoe Canyon Fm.	7/14/2014	caudal vertebra
Crossopholis magnicaudatus	33530 ± 170	1.54	-26.18	Green River Fm.	3/20/2013	Paddlefish "cartilage"
Triceratops horridus	33570 ± 120	1.53	-17.1	Hell Creek Fm.	8/14/2012	horn core bulk bone
ceratopsian	36760 ± 130	1.03	-1.7	Horseshoe Canyon Fm.	7/14/2014	caudal vertebra
Axel wood	39720 ± 270	0.71	-22.2	Buchanan Lake Fm.	5/5/2014	unmineralized
Drumheller wood	40040 ± 160	0.68	-24.1	Horseshoe Canyon Fm.		peat-like
Triceratops horridus	41010 ± 220	0.61	-4.3	Hell Creek Fm.	8/14/2012	horn core bioapatite
Czech wood	48160 ± 330	0.25	-22.7	Boskovice Furrow	2/26/2013	carbonized wood
Captorhinus aguti	49470 ± 510	0.21	-29.7	Admiral Fm.	8/5/2014	vert, jaw, leg

Table 1. Carbon isotope data used to plot Figures 1 and 6 are shown from 14 fossils. Radiocarbon ages were copied from referenced sources without calibration or other normalization. Plus/minus value represents 1σ confidence error margins. Pmc refers to percent modern carbon, a ratio of the fraction of ^{14}C to ^{12}C in the sample to the fraction of ^{14}C to ^{12}C in the international standard (where "modern" means AD 1950, and the absolute radiocarbon standard is a sample of wood from a tree that died in AD 1890). Radiocarbon years in "Before Present" are calculated based on pmc. Radiocarbon analyses also supply ^{13}C isotope results, shown as δ13, which represents the parts of ^{13}C in the sample per thousand parts ^{13}C in an international standard. Negative values, below the standard zero value, are typical for samples of great antiquity.

report herein. These relatively minute differences do not impact the thrust of this present investigation, which targets the three orders of magnitude difference between radiocarbon years (either uncalibrated or calibrated) and conventional "ages."

Fish Fossil Specimens

A fossil osteichthyan of genus *Phareodus* (ADM 0112, Alberta Dinosaur Museum) was obtained in 2011 directly from the Green River Formation (GRF) near Kemmerer, Wyoming. The outcrop on private property where the fish fossil was obtained bears a conventional age assignment of approximately 49 million years. The specimen, seen in Figure 2, had not been prepared after its removal from the "split fish layer," and thus provided a pristine sample for carbon isotope analysis, untouched with any glues, preservatives, or other commercial contaminants. This and similar sites in the GRF have supplied a wide variety of reptilian, avian, and mammalian lagerstatte, famous worldwide for preservation of even soft body parts (Whitmore, 2006), including original vertebrate proteins (Edwards et al., 2011). Portions of the skull, jaw with teeth, and scales were carefully extracted from the shale matrix using clean stainless steel tools directly onto tin foil. The sample was sent to a commercial U.S. radiocarbon facility, which returned 3.87 pMC, or 26,110 ± 60 BP as plotted on Figure 1.

Our chondrichthyan *Crossopholis magnicaudatus* fossil was purchased by the Institute for Creation Research (ICR) at a 2009 Heritage Auction event that supplied original fossils to museums and private parties. Lot number 41304 at auction 6012 is being accessioned by the Institute provisionally as ICR 0111.

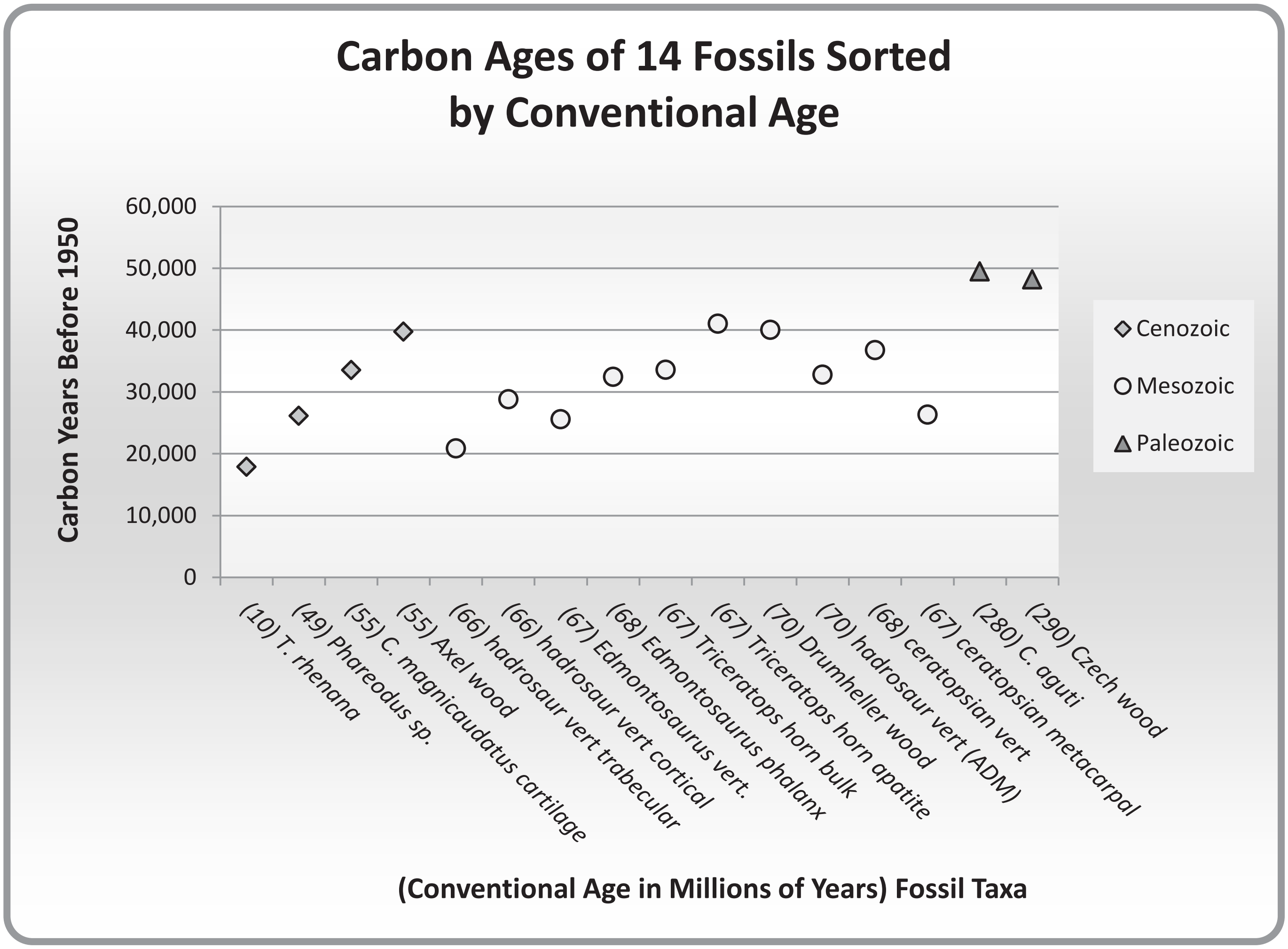

Figure 1. Carbon dates measured by AMS for 14 fossils including seven dinosaur bones lie within approximately 17,850 and 49,470 radiocarbon years. All fossils so far tested returned a carbon age. These carbon ages starkly contrast the approximate conventional ages shown in parentheses for each fossil. "Hadrosaur vert cortical" marks the carbon date for the exterior bone, adjacent to rock matrix, and "hadrosaur vert trabecular" indicates the carbon date for the interior of the same bone (ICR 021). Error bars lie within the graphic markers.

Also exhumed from the famous Fossil Lake region of GRF, this rare, whole paddlefish fossil is 42-¾ inches long and includes darkened, sinewy material that may contain original cartilage. We reasoned that endogenous, little-altered cartilage might retain collagen, the main protein constituent of cartilage. This could preserve ^{14}C if the fossil was deposited fewer than 100,000 years ago. However, the auction description noted, "Where the fine structure of the fins has been lost, sympathetic restoration completes this superb specimen," and we suspected that "sympathetic restoration" may have included modern pigments or coatings as potential contaminants. Thus, we removed a shard from one of the thickest skeletal portions, near the head, with a sterile blade. The shard measured approximately 12 X 3 X 4.5 mm. We indicated on the radiocarbon sample submission form the possibility of bacteria, fungus or oil contamination so that the lab would exercise appropriate decontamination protocols, including an organic solvent and acid-base-acid prewash. The result presented 1.54 pMC, which corresponds to 33,530 ± 170 BP as shown in Figure 1. We note the large disparity between this fossil's carbon age and its standard assigned age of approximately 50 million years.

Wood and Dinosaur Specimens

We obtained a portion of fossil wood of unidentified taxon from a secular 1986 paleontological expedition to Axel Heiberg Island. It came from an upper lignitic coal-bearing member of the Buchanan Lake Formation, Nunavut, Canada. This upper member, assigned an Eocene conventional age, is known for naturally mummified leaf tissues (Shoenhut, 2005), leaf mats, stumps, cones, and seeds (LePage et al., 1991). The fossil wood still had a fresh-looking appearance, was partially pliable, and easily ignited at standard temperature and pressure with a common butane fuel source. These features clearly indicated a lack of mineralization and thus the potential for endogenous woody compounds containing carbon. A core sample of still-brown-colored wood (ADM 0113) was submitted for radiocarbon dating, which reported 0.71 pMC. This corresponds to 39,720 ± 270 BP as Figure 1 indicates.

Next, a whole hadrosaur caudal vertebra (ICR 021, Institute for Creation Research), minus its spinous process, was donated by Mr. Paul Koepp of Dallas, Texas. He acquired it from Stuart Schmidt of Grand River Museum near Lemmon, South Dakota. GRM houses a sizeable collection of dinosaur fossils collected by summer dig groups that Mr. Russ McGlenn of Adventure Safaris and the Twin Cities Creation Science Association organizes on Stuart Ranch property, located approximately 40 miles from Lemmon near Keldron, SD. Abundant disarticulated hadrosaur and ceratopsian fossils occur on the Schmidt ranch, as at other Hell Creek Formation sites, mostly across Montana. Our cylindrical bone was collected by one of McGlenn's groups circa 2006 and retained at Grand River Museum until transfer to ICR in 2012. The interior trabecular bone was plainly not infilled with sediment, and retains its *in vivo* spongy structure, albeit colored almost black, as shown in Figure 3a. This fossil also yielded radiocarbon in amounts well within the detection threshold of the AMS technique at 20,850 ± 90 BP for the interior trabecular portion, and 28,790 ± 100 BP, as shown in Figure 1.

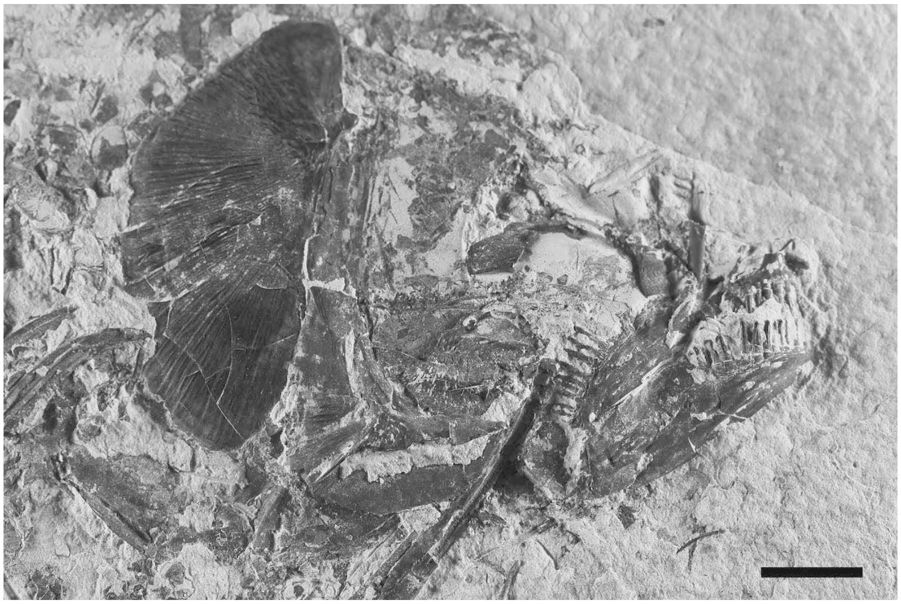

Figure 2. *Phareodus* fossil fish fragments from the Green River Formation, supposedly more than 40 million years old, returned an approximate age of 26,110 radiocarbon years. Jaw and teeth are visible on the right, and the dark oval structure is its operculum. Scale bar equals 10mm.

In 2013 we acquired two hadrosaurid bones from the Lance Formation from a professional fossil dealer in the U.S. with a large personal network of fossil excavators. These bones were observed to be in pristine condition, exactly as found, with no glue present or preservative applied. One hadrosaur caudal vertebra from an *Edmontosaurus* had a very similar color, condition, and lithologic context to the ICR 021 vertebra, and both are pictured in Figure 3. As a kind of sister to the Hell Creek Formation, the Lance Formation is assigned a conventional age of late Cretaceous. The other hadrosaurid bone procured was also from an *Edmontosaurus*, but is a foot phalanx. To provide the purest samples possible for carbon dating, both of these whole bones were split open and material was removed from the center of each using clean stainless steel instruments. The samples were collected directly onto tin foil. No silt or other material was observed within the center of these bones. Furthermore, the bones were not mineralized to any noticeable degree, as seen in Figure 3. The trabecular cavities were still open as in a modern bone of a similar kind, though the fossils exhibited a more brownish color in their interiors. We plotted in Figure 1 the reported carbon ages of these *Edmontosaurus* bone fossils at 25,550 ± 60 for the vertebra, and 32,420 ± 160 BP for the phalanx.

A *Triceratops horridus* horn core (HCTH 00), excavated by Armitage and Anderson as part of the Creation Research Society's iDINO effort, yielded a spectacular whole sheet of soft, fibrillar tissue. The bone's geographic and geologic settings were described in Armitage and Anderson's *Acta Histochemica* paper (Armitage and Anderson, 2013). We reasoned that a fossil containing original fibrillar material must preserve proteinaceous connective tissue and thus possibly endogenous ^{14}C. We note that Armitage and Anderson (2013) expressed surprise over their discovery

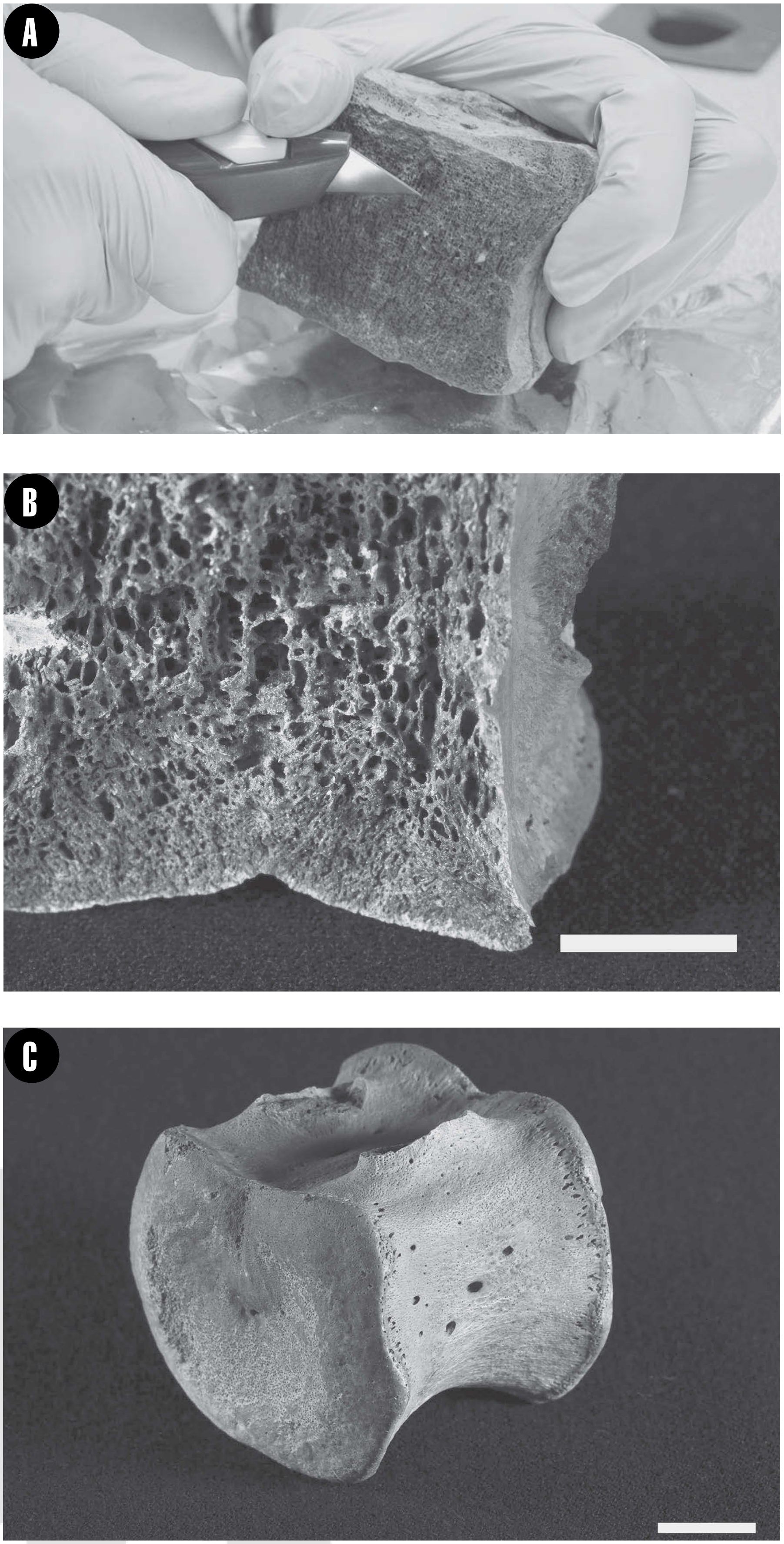

Figure 3 *(left)*. Trabecular bone spaces of these hadrosaur vertebrae are clean and vacant, showing no sedimentary or any other infilling. (a) Darkened core bony material from the center of a freshly split caudal vertebra (ICR 021) from North Dakota Cretaceous Hell Creek Formation yielded approximately 20,850 radiocarbon years. The exterior of this bone dated to approximately 28,790 radiocarbon years. (b) Darkened core bony material from the center of freshly split caudal vertebra from Cretaceous Lance Formation returned an age of approximately 25,550 carbon years. (c) Whole *Edmontosaurus* vertebra (except for spinous processes that are only rarely preserved) prior to fracturing reveals original bone color, indicating no mineralization. Scale bars equal 10mm.

of still-stretchy tissue within this horn bone core given that it "actually had a muddy matrix deeply embedded within it, which became evident when the horn fractured." In this case, the presence of soft tissue contradicted an intuition-based suggestion that recurring hydration accelerates tissue decay. In a similar way, could endogenous radiocarbon persist within a seasonally damp bone sample? See our discussion below for indications that the answer could be affirmative.

A portion of this horn core was submitted to the AMS dating facility. At the suggestion of Mr. Hugh Miller, we requested that the lab separate the bioapatite fraction of the bone for one carbon date test and separately obtain a bulk bone (bioapatite plus any organics including proteins) test result. The bulk bone yielded a uniformitarian carbon age of 33,570 ± 120 BP, and 41,010 ± 220 BP for the bioapatite fraction. The results are shown in Figure 1 as "*Triceratops* horn bulk" and "*Triceratops* horn apatite," respectively. The greater age for our bioapatite fraction resembles the pMC proportions in modern bone fractions. The odds that modern radiocarbon could contaminate fractions of a fossil bone by adding the same proportions of all three carbon isotopes that occur in modern bone should be exceedingly small. However, given that the variation between carbon dates taken from very nearby samples (and as described below, other authors found a range of carbon dates from within the same sample) overlaps the variation between these two horn core fractions, there may be other explanations for these results. We are currently gathering more carbon dates from bone fractions to further test the hypothesis that the pMC difference between fossil bone fractions resembles the pMC difference between modern bone fractions.

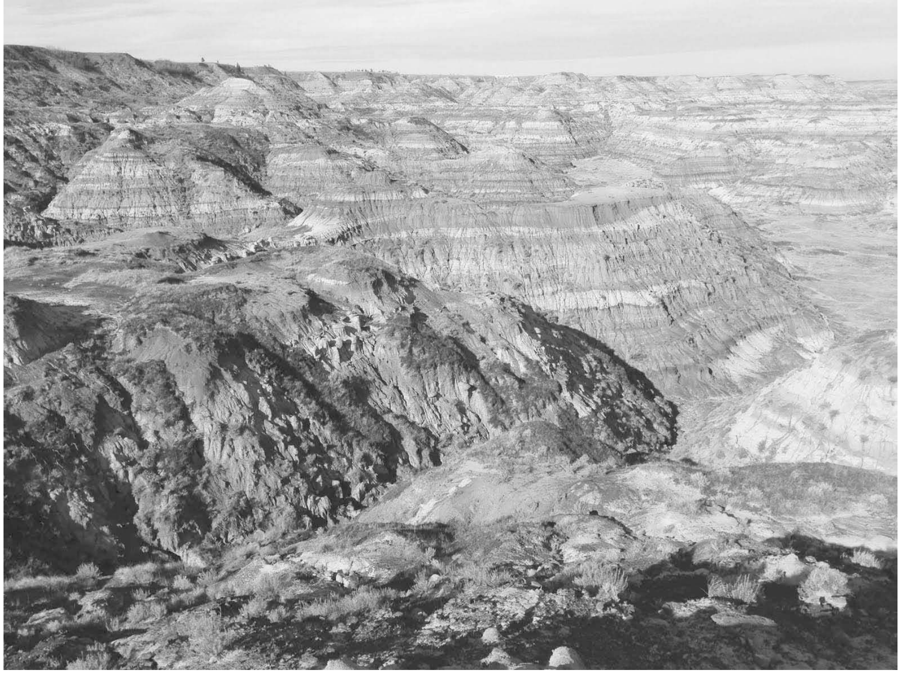

Figure 4. The badlands of Alberta's Horseshoe Canyon Formation contain many dinosaur and other fossils, including our hadrosaurian caudal vertebra, a ceratopsian caudal vertebra, a ceratopsian metacarpal V and a wood sample. Their uniformitarian carbon ages were approximately 32,770; 36,760; 26,300; and 40,040 radiocarbon years, respectively.

Figure 4 shows an exposure of the Late (Upper) Cretaceous Horseshoe Canyon Formation in Alberta, near Drumheller, a site that typifies its badlands topology. We see here exposed layers of shaley sandstone, thin coal seams, carbonaceous shale, and mudstones. Like the Hell Creek and Lance Formations, we associate the deposition of Horseshoe Canyon Formation's layers with the middle-Flood months, when Rising Rocky Mountain terrains temporarily trapped Floodwaters perhaps near the apex of water coverage (Clarey, 2015). Late Flood or residual Flood catastrophism may have eroded then-soft sediments to generate the badlands topology found across northwestern North America. A fresh-looking, semisoft, peat-like sample of wood was discovered in the Horseshoe Canyon Formation by Author Nelson on October 14, 2013. Official disposition was granted on May 5, 2014 by the government of Alberta. The sample for carbon dating was then obtained by removing outer layers of the wood to obtain core portions, which were sent to the radiocarbon facility for dating. Our wood specimen of unknown taxon (ADM 0116) appears as "Drumheller wood" in Figures 1 and 6. Its carbon age was reported as 40,040 ± 160 BP.

We also acquired a hadrosaurian caudal vertebra (ADM 0117), a ceratopsian caudal vertebra (ADM 0118), and a ceratopsian metacarpal V (ADM 0119) directly from their discoverer, who indicated that the bones had never been prepared, glued, or sealed but were exactly as found within the Horseshoe Canyon Formation, from which our wood (ADM 0116) was also obtained. Figure 1 also shows their uniformitarian radiocarbon ages of 32,770 ± 100, 36,760 ± 130, and 26,300 ± 60 BP, respectively.

Lizard and Wood Specimens

We acquired *Captorhinus aguti* fossils to test for the presence of radiocarbon in the Paleozoic erathem. Because of the persistent failure of AMS facilities to consistently find carbon-dead samples—even from Paleozoic sources—for use as machine blanks (see introduction), and based on the assumption that Paleozoic strata represent Flood deposits, radiocarbon is expected. The extinct nondinosaurian reptile *C. aguti* indexes Permian strata and retains a conventional age range of between 280 and 270 million years. We obtained through a professional paleontologist high quality original specimens, including complete foot bones, jaw fragments with teeth, skull fragments, and vertebrae, shown in Figure 5. Its provenance describes an extension into Oklahoma of the Admiral Formation, with most of the Formation's footprint found in Texas. The lab detected 0.21 pMC, corresponding to 49,470 ± 510 BP, shown in Figure 1.

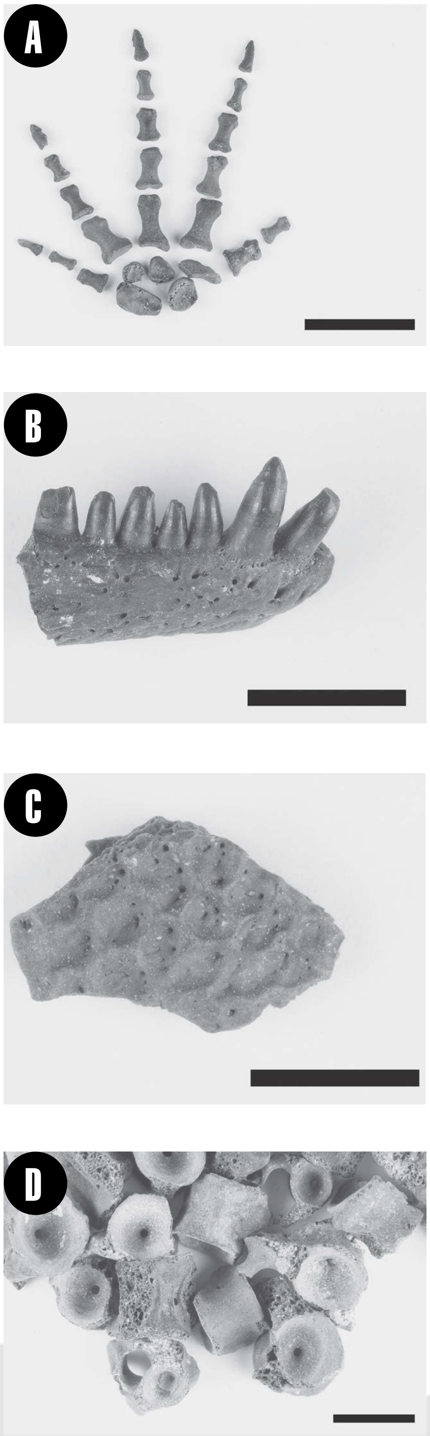

Figure 5. Fragments of *Captorhinus aguti*, an extinct lizard index fossil for the Permian System collectively yielded an age of approximately 49,470 radiocarbon years. (a) Complete foot bones; (b) mandible fragment with teeth; (c) skull fragment; (d) vertebrae. Scale bar in 5a equals 10mm, and 5mm in all others.

The final fossil on our list, and the one holding the oldest conventional age assignment in our collection, is a wood sample obtained from the Boskovice Furrow in the Czech Republic. This wood was carbonized and almost black. It was procured from a professional fossil dealer in the U.S. It had been found ensconced within a small shaley slab that also contains an exquisitely preserved fossil branch of genus *Walchia* (a conifer). Under 60X magnification, it was apparent that the wood had not been glued or sealed with any contaminating substance. Using stainless steel instruments, the wood was carefully extracted directly onto tin foil. Its conventional age at 290 million years stands in stark contrast to its uniformitarian carbon age of 48,160 ± 330 BP. We list this fossil in Figures 1 and 6 as "Czech wood."

Results and Discussion

Consistent Radiocarbon in Fossils

Unexpectedly, all 16 samples submitted for measurement contained C-14. We found measurable amounts of ^{14}C in all 14 of our dinosaur and other fossils. Moreover, we found surprising consistency in these data, which range from approximately 17,850 to 49,470 radiocarbon years as indicated in Figure 1.

Comparison with Published Ages

One way to falsify the hypothesis that our 14 fossils contain endogenous radiocarbon is to compare their pMC measurements to those supplied by other workers who also demonstrated each of their artifacts' provenances. We reasoned that a higher average pMC, indicating a lower average number of carbon years, in our data compared to previously published Phanerozoic fossil carbon dates would implicate ^{14}C contributions from contaminants in our samples. We therefore plotted our ^{14}C data along with ^{14}C ages for mostly Mesozoic fossils in Figure 6.

The single secularly published Cretaceous carbon date came from the early Maastrichtian Ciply Phosphatic Chalk of Southwest Belgium. The mosasaur fossil humerus (IRSNB 1624; Institut Royal des Sciences Naturelles de Belgique) yielded direct evidence of endogenous bone proteins as well as radiocarbon, shown in Figure 6 as "mosasaur." The study authors suggested, but did not show convincing evidence, that bacterial contamination caused their result. In any case, at 24,600 carbon years, it fits well within the range of carbon dates assembled in this report (Lindgren et al, 2011).

A remarkable carbon isotope analysis of a Cambrian fossil from the Burgess Shale of the Canadian Rockies, accompanied by a half dozen techniques used to verify chitin endogenous to the genus *Vauxia* sea sponge, revealed a calibrated carbon age of 43,000 years BC (Ehrlich et al, 2013). The study authors wrote in their paper's supplement that their result, coming as it did from a 505-million-year-old source, "has to be seen as [a] minimum age resulting from contaminations." Similar to Lindgren et al's analysis of a mosasaur, they proposed sources of contamination (bacteria, topsoil, and siderite minerals) but did not test these. Our results can be compared to this data point, shown on Figure 6 as "Burgess Sponge."

Igneous petrologist Andrew Snelling carbon dated fossil wood extracted from the middle Triassic Hawkesbury Sandstone of Queensland (Snelling, 1998). The seed-fern *Dicroidium* is common in the Hawkesbury Sandstone near Sydney at Bunadoon, Australia. Snelling acquired it from quarry workers and tentatively identified it as *Dicroidium*, "probably the wood from the stem of a frond" (Snelling, 1999). Geochron Laboratories near Boston detected radiocarbon in the partly petrified wood. Its corresponding carbon age of 33,720 ± 430 years BP is shown in Figure 6.

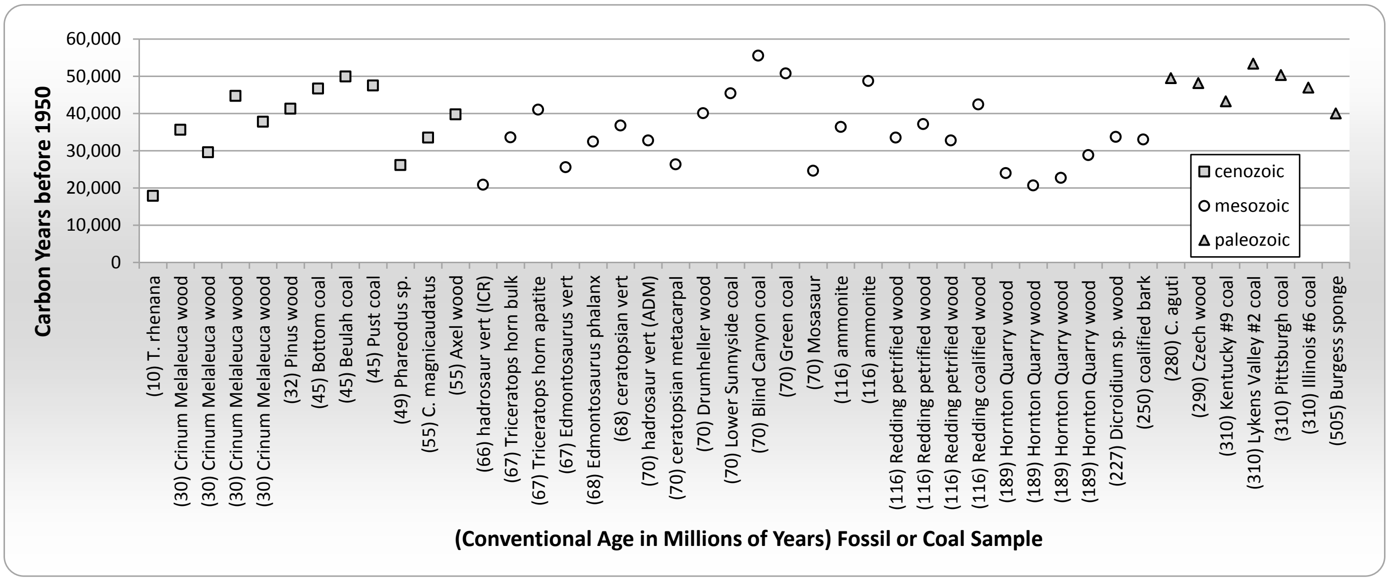

Figure 6. Our 16 dinosaur and other fossil carbon dates mingled with already published AMS carbon dates for fossils plus coal samples are listed in order of conventional age. These 43 results lie within a range of approximately 17,850 and 53,381 radiocarbon years. These data indicate that fossils may systematically exhibit endogenous radiocarbon. As discoveries of radiocarbon in fossil materials increase, arguments of contamination become less plausible. Indeed, if these carbon dates are illegitimate, then the whole carbon dating industry would be held suspect. Error bars lie within the graphic markers.

Snelling also carbon dated wood samples from Australia's Crinum mines (Snelling, 2000a). He carefully documented the provenance, tentatively identifying the fossils as incompletely petrified *Melaleuca* entombed in olivine basalt conventionally aged at 30 Ma. Figure 6 includes all four carbon dates presented in this paper by Snelling, obtained by two different laboratories. He presented carbon dates for wood from a ventilation shaft and one, dated twice, from a drill core. These four samples appear in Figure 6 as "Crinum Melaleuca wood" and derive from the same tertiary basalt flow in Bowen Basin that overlies a Permian sandstone and siltstone.

The upper nine feet of the Marlstone Rock Bed, with outcrops across southern England, contains *Dactylioceras tenuicostatum* ammonite and genus Acrocoelite belemnite fossils that index the sometimes sandy limestone to the early Jurassic System. Nevertheless, Snelling collected and dated four wood samples from this rock at Hornton Quarries, shown in Figure 6 under "Hornton Quarry wood" (Snelling, 2000b). The samples he sent to Geochron yielded 24,005 ± 600, 22,730 ± 170, and 28,820 ± 350 carbon years, and one he sent to a separate lab, ANSTO, yielded 20,700 ± 1,200 carbon years. ANSTO used different standards in reporting error margins, as Snelling describes in his various reports.

Snelling later reported another carbon-dated wood sample to 41,260 ± 540 carbon years. Found near Cripple Creek, Colorado, it was described as having been embedded in lava conventionally dated by the Ar-Ar method at 32 million years old. He wrote, "It can only be concluded, therefore, that the radiocarbon measured by the laboratory must be real *in situ* radiocarbon intrinsic to the original wood, and not contamination of any sort" (Snelling, 2008a). Here we summarize four rationales Snelling used to support his conclusion and then apply his rationales to our results.

1. Laboratory handling could not have introduced nearly enough modern ^{14}C to produce these results because they carefully and rigorously exclude contaminants using procedures such as acid pretreatments and regular calibrations of the accelerator mass spectrometer.
2. Similarly, laboratory treatment in acid removes surface contaminants that may have been introduced by handling in the field. Our fossil bone samples were pretreated with an acid-base-acid prewash procedure that has proven its reliability in many prior experiments and has thus formed the basis of the industry of carbon dating archaeological bone (Aitken, 1990, pp. 86–89).
3. Contamination could have occurred in his wood via post-burial alteration

facilitated by hydrothermal fluid transport of modern radiocarbon sources. However, rigorous demineralization during sample preparation at the carbon dating facility removes these potential contaminants.

4. Contamination of his carbon-dated wood sample might have occurred in the intervening millennia through groundwater percolation. However, the fossil, found deep in a mine, had been located over 3,000 feet below the surface, where groundwater mixing to the extent required to produce the measured amounts of ^{14}C after millions of years would have been very unlikely. Also, the soluble forms of carbon dissolved in groundwater would not interact with the insoluble forms of carbon in the fossil.

In considering our 16 samples, we note that groundwater interaction should have been much more likely since our fossils were not taken from such great depths. Does this falsify our results? In response we note that the soluble versus insoluble forms of carbon remain incompatible regardless of depth or hydration levels. For example, carbon-containing collagen protein is insoluble in water and has not been shown to readily react with dissolved carbonates.

Interestingly, the moisture surrounding the iDINO *Triceratops* horn core fossil showed it had been exposed to ground water when it was first uncovered—indeed, the base of the horn was exposed at the surface prior to excavation (K. Anderson, personal communication). Our hadrosaur vertebra was also removed from very near the ground surface when discovered, our other fossils came from sites better protected from groundwater, and yet the carbon dates from all fossils fell within a similar range, as shown in figures 1 and 6. Also, we have a preliminary indication that the most sequestered sections of our fossils, for example interior trabecular bone scrapings from the hadrosaur vertebra (ICR 021), and the exposed exterior portions ("hadrosaur vert cortical" in Figure 1) both present consistent radiocarbon ages. If percolation contaminated these fossils, their exteriors should show lower carbon ages than their interiors, but we did not find this. Plus, as noted above, studies comparing the ratios of ^{14}C to those in modern bone will further test the effects of groundwater.

Last, Snelling described in detail the geologic setting for a fossil ammonite collected near Redding, California, the shell of which yielded two additional carbon dates: 36,400 and 48,710 carbon years (Snelling, 2008b).

Overall we find that our 16 carbon dates fall within a range that closely approximates that of similar data already published, consistent with the hypothesis that our dinosaur bone and other fossil materials contain endogenous ^{14}C. In sum, we note that Snelling's same four reasons point to a similar conclusion for our 16 carbon-dated dinosaur and other fossils.

We also included in our Figure 6 the ten carbon dates for carefully extracted coal samples that the RATE team published (Baumgardner, 2005). The coals did show higher carbon ages on average than fossil carbon ages from comparable erathems, but those coal carbon ages did overlap with some fossil carbon ages, which in turn overlapped other carbon ages. These overlaps seem to suggest natural, endogenous fluctuations in radiocarbon amounts per sample instead of a systematic bias arising from contamination. Future research could explore possible reasons for higher average carbon ages in coals as compared to fossils.

After comparing these data in attempts to falsify the hypothesis of authigenic radiocarbon, we noticed that we had compiled carbon dates for fossils from all three erathems, enabling us to compare carbon dates to a broad range of conventional age assignments. The pMC averages and ranges from our combined data set are displayed according to erathems in Figure 7. We find that although pMC in fossils does not significantly differ between erathems, they infer carbon ages that are orders of magnitude younger than conventional age assignments for these fossils. This result is consistent with the hypothesis that even geologic strata described as Paleozoic, and including the Mesozoic, were deposited in one year only thousands of years ago during Noah's Flood.

Comparing Laboratories

We considered the possibility that error could arise from a given carbon-dating facility, for example by operator error on a given day. Our 16 results from a carbon isotope facility that specializes in bone tissue combine with similar detections of radiocarbon in Phanerozoic fossils from Geochron Laboratories in Massachusetts, the IsoTrace Radiocarbon Laboratory at the University of Toronto in Ontario, the Australia Nuclear Science and Technology Organisation (ANSTO), and the Jena AMS system at the Max-Planck Institut für Biogeochemie. The most parsimonious explanation for five different laboratories detecting radiocarbon in ancient fossils seems to be that these fossils contain endogenous ^{14}C.

Comparing Localities

According to the Creation-Flood model, water covered the whole globe recently, enabling us to interpret the global occurrences of fossils largely as resulting from this cataclysm. Given such universally recent deposition, ^{14}C might be detected worldwide, and our results confirm this. The geographical settings of radiocarbon-containing fossils and coals from Figure 6 include:

> Adendorf, Germany; Alberta, Canada; Arizona; Czech Republic; Mons, Belgium; California; Colorado; Illinois; Kentucky; Montana; North Dakota; Nunavut, Canada; Oklahoma; Pennsylvania; Ratley, England; Sydney, Australia; Queensland, Australia; Texas; Utah; and Wyoming.

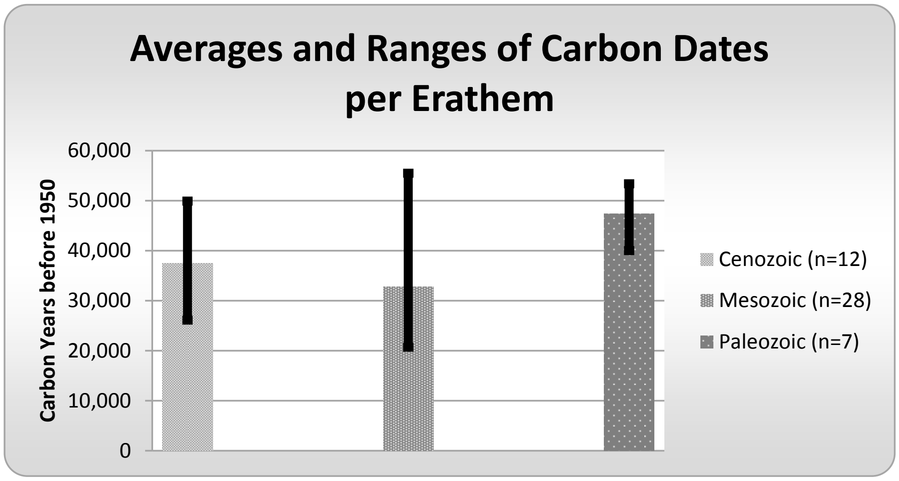

Figure 7. Histogram bar heights represent average carbon dates of Figure 6 samples from each erathem. Solid black lines represent carbon date ranges for each erathem. Mesozoic samples show the widest range probably because they include the highest number of samples. Although our youngest conventionally aged fossil also showed the youngest carbon age, and our oldest conventionally aged fossils showed the oldest carbon ages, these are not enough data to resolve a relative time trend across erathems. Future research, including additional carbon dates and statistical analyses might reveal new trends in fossil carbon dates.

This wide distribution makes it difficult to assert that radiocarbon in fossils arises from a local source of contamination, and suggests that ^{14}C might be found at any location worldwide where a fossil occurs. More data from additional sites will help generate an accurate picture of the distribution of ^{14}C, but thus far these data are consistent with the Creation-Flood model.

Conclusions

On the basis of repeated observations of original tissues found in dinosaur and other bones, and on the basis of prior researchers' difficulties in finding a reliable source of carbon-dead earth material to calibrate highly sensitive AMS systems, we tested 16 fossil samples for the presence of ^{14}C. Our fossils came from seven dinosaurs, including two dates from the iDINO project's *Triceratops* horn core, which had a spectacular whole sheet of soft, fibrillar tissue, and two dates from a hadrosaur vertebra, a fruit, one cartilaginous fish, one bony fish, three wood samples, and one extinct lizard. We sought to falsify the hypothesis that this radiocarbon originated from our fossil material first by comparing our amounts of measured radiocarbon to those already published, finding general concordance between our data and already published data. Second, all samples, regardless of geographic and even stratigraphic provenance, showed detectable ^{14}C within a total age range spanning orders of magnitude smaller than the conventional age range for these fossils. In addition, some of our fossil material was not extracted from as great a depth as were certain previously published carbon-dated fossils like wood from Californian and Australian mine shafts, and yet both settings revealed very comparable age averages and ranges. Third, five different laboratories all detected radiocarbon in fossils from locations worldwide, arguing against contamination by poor lab practice or by any local anomaly. These observations are not consistent with contamination. Finally, similar levels of radiocarbon were recovered from all three erathems, consistent with the hypothesis that all three erathems contain fossils deposited during Noah's year-long Flood only thousands of years ago. Additional tests of more material would add more data for further analyses. In particular, we plan to extend our single comparison of the ^{14}C ratio between bioapatite and bulk bone fractions to investigate whether or not dinosaur material trends toward mimicking similar carbon isotope ratios as found in modern bone. Additional carbon dates could also reveal relative age trends across erathems.

References

Aitken, M.J. 1990. *Science-based Dating in Archaeology*. Longman, London, UK.

Armitage, M.H., and K.L. Anderson. 2013. Soft sheets of fibrillary bone from a fossil of the supraorbital horn of the dinosaur *Triceratops horridus*. *Acta Histochemica* 116(6): 603–608.

Arnold, J.R., and W.F. Libby. 1949. Age determinations by radiocarbon content: checks with samples of known age. *Science* 110(2869): 678–680.

Baumgardner, J., A.A. Snelling, D.R. Humphreys, and S.A. Austin. 2003. Measurable ^{14}C in fossilized organic materials: confirming the young earth Creation-Flood model. In Ivey, H. (editor), *Proceedings of the Fifth International Conference on Creationism*, pp. 127–142. Creation Science Fellowship, Pittsburgh, PA. (http://www.icr.org/article/young-earth-creation-flood-14c/)

Baumgardner, J. 2005. ^{14}C evidence for a recent global flood and a young earth. In Vardiman, L., A.A. Snelling, and E.F. Chaffin (editors), *Radioisotopes and the*

Age of the Earth: Results of a Young-Earth Creationist Research Initiative, pp. 587–630. Institute for Creation Research, El Cajon, CA, and Creation Research Society, Chino Valley, AZ. (http://www.icr.org/article/carbon-14-evidence-for-recent-global/)

Cherkinsky, A. 2009. Can we get a good radiocarbon age from "bad bone"? Determining the reliability of radiocarbon age from bioapatite. *Radiocarbon* 51(2): 647–655.

Clarey, T. 2015. The Hell Creek Formation: the last gasp of the pre-Flood dinosaurs. *Creation Research Society Quarterly* 51:286–298.

Dahmer, L., D. Kouznetsov, A. Ivenov, J. Hall, J. Whitmore, G. Detwiler, and H. Miller. 1990. Report on chemical analysis and further dating of dinosaur bones and dinosaur petroglyphs. In Walsh, R.E., and C.L. Brooks (editors), *Proceedings of the Second International Conference on Creationism, Vol. II*, pp. 371–374. Creation Science Fellowship, Pittsburgh, PA.

Edwards, N.P., H.E. Barden, B.E. van Dongen, P.L. Manning, P.L. Larson, U. Bergmann, W.I. Sellers, and R.A. Wogelius. 2011. Infrared mapping resolves soft tissue preservation in 50 million year-old reptile skin. *Proceedings of the Royal Society B* 7:3209–3218.

Ehrlich, H., J.K. Rigby, J.P. Botting, M.V. Tsurkan, C. Werner, P. Schwille, Z. Petrášek, A. Pisera, P. Simon, V.N. Sivkov, D.V. Vyalikh, S.L. Molodtsov, D. Kurek, M. Kammer, S. Hunoldt, R. Born, D. Stawski, A. Steinhof, V.V. Bazhenov, and T. Geisler. 2013. Discovery of 505-million-year old chitin in the basal demosponge *Vauxia gracilenta*. *Scientific Reports* 3:3497.

Hebert, J. 2013. Rethinking carbon-14 dating: what does it really tell us about the age of the earth? *Acts & Facts* 42(4): 12–14.

International Chronostratigraphic Chart. 2014. International Commission on Stratigraphy. http://www.stratigraphy.org/index.php/ics-chart-timescale

LePage, B.A., and J.F. Basinger. 1991. Early Tertiary Larix from the Buchanan Lake Formation, Canadian Arctic Archipelago, and a consideration of the phytogeography of the genus. In Christie, R.L., and N.J. McMillan (editors), *Tertiary Fossil Forests of the Geodetic Hills, Axel Heiberg Island, Arctic Archipelago*, GSC Bulletin 403, pp. 67–82. Geological Survey of Canada, Ottawa, ON.

Lindgren, J, P.Uvdal, A. Engdahl, A.H. Lee, C. Alwmark, K-E. Bergquist, E. Nilsson, P. Ekström, M. Rasmussen, D.A. Douglas, M.J. Polcyn, and L.L. Jacobs. 2011. Microspectroscopic evidence of Cretaceous bone proteins. *PLoS ONE* 6(4): e19445. (http://www.plosone.org/article/info%3Adoi%2F10.1371%2Fjournal.pone.0019445)

Lot 41304; Rare fossil paddlefish. 2009. Heritage Auction. http://fineart.ha.com/itm/fossils/fish/rare-fossil-paddlefish/a/6012–41304.s (accessed November 2014).

Nadeau, M.-J., P.M. Grootes, A. Voelker, F. Bruhn, A. Duhr, and A. Oriwall. 2001. Carbonate ^{14}C background: does it have multiple personalities? *Radiocarbon* 43:2A, 169–176.

Shoenhut, K. 2005. Ultrastructural preservation in Middle Eocene Metasequoia leaf tissues from the Buchanan Lake Formation. In LePage, B.A., C.J. Williams, and H. Yang (editors), *Topics in Geobiology: The Geology and Biology of Metasequoia, Part II* 22:219–252. Springer, Dordrecht, Netherlands.

Snelling, A.A. 1998. Stumping old-age dogma: radiocarbon in an 'ancient' fossil tree stump casts doubt on traditional rock/fossil dating. *Creation* 20(4): 48–51. (http://creation.com/stumping-old-age-dogma)

Snelling, A.A. 1999. Dating dilemma: fossil wood in 'ancient' sandstone. Creation.com. http://creation.com/dating-dilemma-fossil-wood-in-ancient-sandstone (accessed February 16, 2015).

Snelling, A.A. 2000a. Conflicting 'ages' of Tertiary basalt and contained fossilised wood, Crinum, Central Queensland, Australia. *Journal of Creation* (*CENTJ*) 14(2): 99–122. (http://creation.com/images/pdfs/tj/j14_2/j14_2_99–122.pdf)

Snelling, A.A. 2000b. Geological conflict: young radiocarbon date for ancient fossil wood challenges fossil dating. *Creation*. 22(2): 44–47. (http://creation.com/geological-conflict)

Snelling, A.A. 2008a. Radiocarbon in "ancient" fossil wood. *Acts & Facts* 37(1): 10. (http://www.icr.org/article/radiocarbon-ancient-fossil-wood/)

Snelling, A.A. 2008b. Radiocarbon ages for fossil ammonites and wood in Cretaceous strata near Redding, California. *Answers Research Journal* 1 (2008): 123–144. (https://answersingenesis.org/geology/carbon-14/radiocarbon-ages-fossils-cretaceous-strata-redding-california/)

Whitmore, J. 2006. The geologic setting of the Green River Formation. *Journal of Creation* 20(1): 72–78. (http://creation.com/the-geologic-setting-of-the-green-river-formation)

Addendum

More background surrounding the long-standing debate over the usefulness for secular archaeology of ^{14}C dates from bone apatite has come to the authors' attention since we submitted this paper. Zazzo (2014) showed evidence that apatite suffers from isotopic exchange of modern carbonates in the presence of groundwater, leading to younger radiocarbon ages for apatite than expected. He and Cherkinsky (2009) noted that ^{14}C introduced in this manner cannot be removed with pretreatments. However, Cherkinsky and Chataigner (2010) showed that some apatite fractions yielded carbon ages older than those of collagen fractions from the same bone. Even so, we calculate that discordance between collagen/charcoal/eggshell radiocarbon dates and their corresponding apatite dates from material older than 10,900 RCY in Zazzo (2014) averages 19.75%, implying that even in samples where isotope exchange may have rejuvenated apatite carbonates, most of the measured ^{14}C is endogenous. These considerations do not appear to significantly impact our major conclusions, but provide direction for future research.

References

Cherkinsky, A. 2009. Can We Get a Good Radiocarbon Age from “Bad Bone?” Determining the Reliability of Radiocarbon Age from Bioapatite. *Radiocarbon* 51(2): 647–655.

Cherkinsky, A., and Chataigner, C. 2010. ^{14}C Ages of Bone Fractions from Prehistoric Armenian Sites. *Radiocarbon* 52(2–3): 569–577.

Zazzo, A. 2014. Bone and enamel carbonate diagenesis: A radiocarbon prospective. *Palaeogeography, Palaeoclimatology, Palaeoecology* 416 (2014) 168–178.

Letters to the Editor

The policy of the editorial staff of CRSQ *is to allow letters to the editor to express a variety of views. As such, the content of all letters is solely the opinion of the author, and does not necessarily reflect the opinion of the* CRSQ *editorial staff or the Creation Research Society.*

Snowmass Fossils

I want to commend young Andrew St. Marie for his interesting and informative article, “Evidence for the Recent Existence of Mammoths and Mastodons,” in the Fall 2014 edition of *CRSQ*. While I have never met Andrew, we share a fondness for fossils and creationist paleontology and are acquainted via e-mail.

I’m honored that Andrew quoted me in his paper about the Snowmass Fossil Site. However, I’d like to make one correction. In citing my (unpublished) article, “Fresh Look (and Smell) of Snowmass Fossils Astonish Evolutionists,” Andrew mentioned that a date of 50,000–150,000 years was assigned to the fossils because they contained no measurable C-14. In reality, the carbon-14 dates obtained were *dismissed* by the scientists involved as “dead” or “unreliable.” This likely occurred for a couple of reasons: (1) Despite C-14 testing results of 43,000–58,000 ya—most near C-14’s commonly accepted accuracy limit of 50,000 years—the age range was probably considered too recent and too narrow for the geological setting. (2) Some of the *older* dates came from fossils in the *upper* layers, while some of the *younger* dates came from those in *lower* strata.[1] Thus, the search was on for “better dates,” using other testing methods, including Optically Stimulated Luminescence and Uranium-Series Dating. And (surprise!) they indeed confirmed the preassigned age range of 50,000–150,000 years.

The Snowmastodon Project, as it was named, was originally touted as “one of the most significant fossil discoveries ever made in Colorado,” by the Denver Museum of Nature & Science (DMNS). Interestingly, however, the final reports from its “international team of scientists” were released without fanfare in the November 2014 issue of *Quaternary Research*. Maybe this is because the findings continue to provide conflicting data about the Snowmass fossil site that don’t match up with evolutionary expectations, much less aid our understanding of “global climate change,” one of the project’s stated goals.

Terry P. Beh

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[1] These data are from a February 16, 2011, meeting of the Colorado Scientific Society at the Colorado School of Mines, where an initial public report on *The Snowmass Fossil Discoveries* was given by paleontologist Dr. Kirk Johnson, Chief Curator at DMNS, and USGS geologist Dr. Jeff Pigati.

Christian Theism Versus Pantheism

We find the scholarly essay by Reed and Klevberg, "Beyond 'Origin & Operation' Science, Part II: An Alternative" (Reed and Klevberg, 2014), brimming with insightful opinions, and we thank the authors for presenting these views. We suspect that the category of "Origin Science" probably developed more because of the considerable progress creationist scientists made in the second half of the twentieth century, uncovering evidences supporting biblical creation, as presented in this journal, than as a reaction to the Arkansas and Louisiana creation trials, which the authors suggest. Nevertheless, they argue correctly that the term 'origin science' can be a tacit surrender to positivism if it does not acknowledge the metaphysical issues, the worldview that is involved. For a Christian, the metaphysical issues find their sole answer in revelation.

While the efforts of Reed and Klevberg to clarify the roles and boundaries of science, history, and philosophy are laudable, we submit that the issue lies deeper. Until creationists clearly see the core conflict, clarifying misunderstandings and rearranging categories won't accomplish that which is sorely needed. It is our conviction that the core, underlying conflict is entirely religious: Christian theism versus pantheism. The "war" is not between science and religion; it's their religion versus ours!

Pantheism is the foundation of modern natural science. Evolution is merely the outworking of a pantheist view of nature. Of course, no scientist today will acknowledge pantheism as his religion in the way that a creationist confesses Christianity, but he's a pantheist nevertheless, not atheist. Atheism doesn't cut it; it doesn't explain the metaphysical issues, the ultimate issues. Nor is he a secularist. It's impossible to be neutral regarding ultimates. Secularism is a dodge, a clever strategy by which to hide one's secret views, which invariably are pantheistic.

Pantheists don't have clear affirmations regarding their "deity." They may believe in an intelligent "force" or energy in the universe, or an impersonal "being"—not a Being as theists know God, but one that fashioned everything there is in the universe so that everything that exists is identical to and is in essence that "being." Pantheists hold that all the cosmos is an outworking of that nonpersonal, non-Being, which they worship by means of naturalism. If a pantheist "statement of faith" could be formulated, we suggest, following Robert Brow (Brow, 1966, p. 82): (1) There is no supernatural Being that is outside of nature; and (2) meaning can be found in aligning ourselves with the principle behind nature, namely evolutionary progress.

Not all Christians know their theology, and not all Christians sense the presence of God in their lives. Even more so, because their god is indefinite, do most pantheists fail to perceive or understand their faith. Yet faith it is! Pantheism is what allows the absurdities of nontheism to be overcome. How else can evolutionists insist that matter can come into existence out of nothing, that there can be the near-universal appearance of design in nature without actual design, and that teleology and information systems can exist without prior inteligent ordering? Why else do they bristle whenever their dogma is questioned? It's the commitment to pantheism that enables scientists to adhere to evolution in the face of withering challenges by creationists. As philosopher and zoologist Michael Ruse wrote, "Evolution is a religion. This was true of evolution in the beginning, and it is true of evolution still today" (Ruse, 2000).

Christians have so organized their thinking around theism that they cannot readily grasp how pantheists can believe in a nonpersonal god (though they may not admit to such a god, not even one with a small *g*), one that comprises all of nature and that imbues nature with the ability to give rise to life and order and complexity. No one can live without some presuppositional understanding of ultimates. (It's how our Creator God made us.) For example, why does anything exist? Where did I come from? And what meaning does life have? Does life have a goal or purpose? What is right and good? However vague the understanding may be in the minds of contemporary scientists, they all have to begin with basics, with first things. Nontheism therefore devolves (in the Western world anyway) into the only alternative: an unstructured religion—pantheism.

Einstein wrote in one of his letters (Temple of Nature, n.d.), "We followers of Spinoza see our God in the wonderful order and lawfulness of all that exists and in its soul as it reveals itself in man and animal." And in a letter in 1954, he wrote, "If something is in me which can be called religious then it is the unbounded admiration for the structure of the world as far as our science can reveal it" (Wikiquote, n.d.). He believed in the impersonal god of pantheism. We submit that pantheism is the view of nature, indeed of all reality, that is prevalent in the West today. One could reasonably argue further that pantheism is the established religion of the United States.

The importance of understanding that we are engaged in an epic religious conflict is that young people in the church can be taught that the views of science they will be exposed to in schools and colleges are at their core this antitheistic religion, pantheism; it's a *religion* that is being forced onto them in their science courses. The academic and scientific-technical people are our culture's "priests," teaching us pantheistic religion. The church today is paralyzed, partly because confidence in the Scriptures has been lost, and the revival that is so desperately needed could result from a fresh awareness that the underlying issue is, fundamentally, a war of religions. Understanding the role of pantheism also would provide the needed rebuttal when a creationist scientist such as Mark Armitage is expelled from a faculty position, or when creationist scientists are passed over for tenure or for grants. And theistic evolutionists should realize that they are engaged in a syncretistic program similar to and as abominable as was occurring during the centuries of the Northern Kingdom.

What gives the lie to pantheism? Revelation. Since the beginning of history, the cosmic conflict has been the Word of God versus the ideas of man. Which will we believe and stake our lives and reputations on? Reason says revelation: The Word of God is a self-attesting authority of supernatural origin. Pantheism (and other religions), in contrast, is based on nothing more authoritative than human speculation.

Kitzmiller v. Dover School Board found that science is not religious (Wikipedia, n.d.). That flawed decision unfortunately was not appealed, only because the citizens of Dover, Pennsylvania, elected a new school board, one that had no stomach for further embarrassment in the national media. We are not arguing here that science is religious but rather that scientists are religious, and their religion is pantheism, and that evolution is the outworking of that religion. And the "-isms" that Reed and Klevberg discuss—naturalism, positivism, and empiricism—are the demands that pantheism makes of its adherents.

Respectfully,
Merrill A. Cohen, MD
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References

Brow, R. 1966. *Religion: Origins and Ideas.* InterVarsity Press, Chicago, IL.

Reed, J.K., and P. Klevberg. 2014. Beyond origin and operation science, part II: an alternative. *Creation Research Society Quarterly* 51:31–39.

Ruse, Michael. "Saving Darwinism from the Darwinians," *National Post*, May 13, 2000, p. B3.

Temple of Nature. n.d. http://pantheist.weebly.com (accessed January 1, 2015).

Wikipedia. n.d. Kitzmiller v. Dover Area School District. http://en.wikipedia.org/wiki/Kitzmiller_v._Dover_Area_School_District (accessed January 1, 2015).

Wikiquote. n.d. Albert Einstein. http://en.wikiquote.org/wiki/Albert_Einstein (accessed January 1, 2015).

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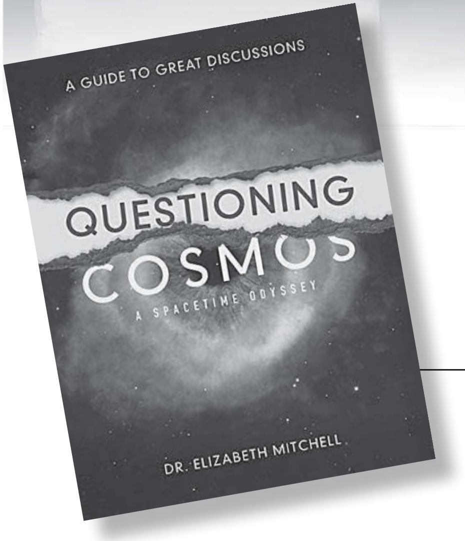

Questioning Cosmos

by Elizabeth Mitchell

Answers in Genesis, Hebron,
KY, 2014, 94 pages, $10.00

Author Mitchell is a retired medical doctor and wife of AIG physician and speaker Tommy Mitchell. In this book the author critiques the summer 2014 television series *Cosmos—A Spacetime Odyssey*, narrated by astronomer Neil deGrasse Tyson. This documentary is an updated version of the popular 1980 *Cosmos* series by Carl Sagan. Since media programs including this new *Cosmos* series have very short shelf lives, the lasting value of a detailed book analysis is uncertain. However, this critique may serve to counter the inevitable packaging and educational distributing of *Cosmos*.

The first paragraph of the book distinguishes between historical and observational science, a major theme in the 2013 debate between Ken Ham and Bill Nye. Secular science does not recognize any fundamental difference between current experimentation and historical analysis. It is implied on page 6 that the scientific method is limited to observational science, a position that would seem to invalidate fields such as paleontology. The reader can make his or her own judgment call on this viewpoint.

Each of the 13 Cosmos episodes is analyzed with a question-answer format with 141 questions total. The included topics emphasized by Neil Tyson include the big bang, origin of life, new planets, Oort comet cloud, star formation, and life in space. It would be interesting to watch the *Cosmos* series with this book in hand to provide commentary. The book pulls no punches with several technical entries. These include Okazaki DNA fragment (p. 17) and the Hertzsprung-Russell star diagram (p. 59).

There are minor weaknesses in the book. The written font within the figure on page 5 is unreadably small. The light wave pattern on page 40 is drawn with confusing, unequal amplitudes. More seriously, there appears to be confusion between magnetic and electric fields. Instead of the actual electric field, magnetic field lines are shown surrounding a Duracell battery.

The paperback book is 8½ x 11 inches in size with many black/white and color pictures. No subject index is provided.

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Instructions to Authors

Submission

Electronic submissions of all manuscripts and graphics are preferred and should be sent to the editor of the *Creation Research Society Quarterly* in Word, WordPerfect, or Star-Office/Open Office (see the inside front cover for address). Printed copies also are accepted. If submitting a printed copy, an original plus two copies of each manuscript should be sent to the editor. The manuscript and copies will not be returned to authors unless a stamped, self-addressed envelope accompanies submission. If submitting a manuscript electronically, a printed copy is not necessary unless specifically requested by the *Quarterly* editor. Manuscripts containing more than 35 pages (double-spaced and including references, tables, and figure legends) are discouraged. An author who determines that the topic cannot be adequately covered within this number of pages is encouraged to submit separate papers that can be serialized.

All submitted manuscripts will be reviewed by two or more technical referees. However, each section editor of the *Quarterly* has final authority regarding the acceptance of a manuscript for publication. While some manuscripts may be accepted with little or no modification, typically editors will seek specific revisions of the manuscript before acceptance. Authors will then be asked to submit revisions based upon comments made by the referees. In these instances, authors are encouraged to submit a detailed letter explaining changes made in the revision, and, if necessary, give reasons for not incorporating specific changes suggested by the editor or reviewer. If an author believes the rejection of a manuscript was not justified, an appeal may be made to the *Quarterly* editor (details of appeal process at the Society's web site, www.creationresearch.org).

Authors who are unsure of proper English usage should have their manuscripts checked by someone proficient in the English language. Also, authors should endeavor to make certain the manuscript (particularly the references) conforms to the style and format of the *Quarterly*. Manuscripts may be rejected on the basis of poor English or lack of conformity to the proper format.

The *Quarterly* is a journal of original writings, and only under unusual circumstances will previously published material be reprinted. Questions regarding this should be submitted to the Editor (CRSQeditor@creationresearch.org) prior to submitting any previously published material. In addition, manuscripts submitted to the *Quarterly* should not be concurrently submitted to another journal. Violation of this will result in immediate rejection of the submitted manuscript. Also, if an author uses copyrighted photographs or other material, a release from the copyright holder should be submitted.

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Manuscripts shall be computer-printed or neatly typed. Lines should be double-spaced, including figure legends, table footnotes, and references. All pages should be sequentially numbered. Upon acceptance of the manuscript for publication, an electronic version is requested (Word, WordPerfect, or Star-Office/Open Office), with the graphics in separate electronic files. However, if submission of an electronic final version is not possible for the author, then a cleanly printed or typed copy is acceptable.

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1. Title page. This page should contain the title of the manuscript, the author's name, and all relevant contact information (including mailing address, telephone number, fax number, and e-mail address). If the manuscript is submitted by multiple authors, one author should serve as the corresponding author, and this should be noted on the title page.

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3. Introduction. The introduction should provide sufficient background information to allow the reader to understand the relevance and significance of the article for creation science.

4. Body of the text. Two types of headings are typically used by the *CRSQ*. A major heading consists of a large font bold print that is centered in column, and is used for each major change of focus or topic. A minor heading consists of a regular font bold print that is flush to the left margin, and is used following a major heading and helps to organize points within each major topic. Do not split words with hyphens, or use all capital letters for any words. Also, do not use bold type, except for headings (italics can be occasionally used to draw distinction to specific words). Italics should not be used for foreign words in common usage, e.g., "et al.", "ibid.", "ca." and "ad infinitum." Previously published literature should be cited using the author's last name(s) and the year of publication (ex. Smith, 2003; Smith and Jones, 2003). If the citation has more than two authors, only the first author's name should appear (ex. Smith et al., 2003). Contributing authors should examine this issue of the *CRSQ* or consult the Society's web site for specific examples as well as a more detailed explanation of manuscript preparation. Frequently-used terms can be abbreviated by placing abbreviations in parentheses following the first usage of the term in the text, for example, polyacrylamide gel electrophoresis (PAGE) or catastrophic plate tectonics (CPT). Only the abbreviation need be used afterward. If numerous abbreviations are used, authors should consider providing a list of abbreviations. Also, because of the variable usage of the terms “microevolution” and “macroevolution,” authors should clearly define how they are specifically using these terms. Use of the term “creationism” should be avoided. All figures and tables should be cited in the body of the text, and be numbered in the sequential order that they appear in the text (figures and tables are numbered separately with Arabic and Roman numerals, respectively).

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Lipman, E.A., B. Schuler, O. Bakajin, and W.A. Eaton. 2003. Single-molecule measurement of protein folding kinetics. *Science* 301:1233–1235.

Margulis, L. 1971a. The origin of plant and animal cells. *American Scientific* 59:230–235.

Margulis, L. 1971b. *Origin of Eukaryotic Cells*. Yale University Press, New Haven, CT.

Hitchcock, A.S. 1971. *Manual of Grasses of the United States*. Dover Publications, New York, NY.

Walker, T.B. 1994. A biblical geologic model. In Walsh, R.E. (editor), *Proceedings of the Third International Conference on Creationism* (technical symposium sessions), pp. 581–592. Creation Science Fellowship, Pittsburgh, PA.

7. Tables. All tables cited in the text should be individually placed in numerical order following the reference section, and not embedded in the text. Each table should have a header statement that serves as a title for that table (see a current issue of the *Quarterly* for specific examples). Use tabs, rather than multiple spaces, in aligning columns within a table. Tables should be composed with *14-point type* to insure proper appearance in the columns of the *CRSQ*.

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Submission of letters regarding topics relevant to the Society or creation science is encouraged. Submission of letters commenting upon articles published in the *Quarterly* will be published two issues after the article’s original publication date. Authors will be given an opportunity for a concurrent response. No further letters referring to a specific *Quarterly* article will be published. Following this period, individuals who desire to write additional responses/comments (particularly critical comments) regarding a specific *Quarterly* article are encouraged to submit their own articles to the *Quarterly* for review and publication.

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All book reviews should be submitted to the book review editor, who will determine the acceptability of each submitted review. Book reviews should be limited to 1000 words. Following the style of reviews printed in this issue, all book reviews should contain the following information: book title, author, publisher, publication date, number of pages, and retail cost. Reviews should endeavor to present the salient points of the book that are relevant to the issues of creation/evolution. Typically, such points are accompanied by the reviewer’s analysis of the book’s content, clarity, and relevance to the creation issue.

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Creation Research Society

History—The Creation Research Society was organized in 1963, with Dr. Walter E. Lammerts as first president and editor of a quarterly publication. Initially started as an informal committee of 10 scientists, it has grown rapidly, evidently filling a need for an association devoted to research and publication in the field of scientific creation, with a current membership of over 600 voting members (graduate degrees in science) and about 1000 non-voting members. The *Creation Research Society Quarterly* is a peer-reviewed technical journal. It has been gradually enlarged and modified, and is currently recognized as one of the outstanding publications in the field. In 1996 the *CRSQ* was joined by the newsletter *Creation Matters* as a source of information of interest to creationists.

Activities—The Society is a research and publication society, and also engages in various meetings and promotional activities. There is no affiliation with any other scientific or religious organizations. Its members conduct research on problems related to its purposes, and a research fund and research center are maintained to assist in such projects. Contributions to the research fund for these purposes are tax deductible. As part of its vigorous research and field study programs, the Society operates The Van Andel Creation Research Center in Chino Valley, Arizona.

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2. All basic types of living things, including humans, were made by direct creative acts of God during the Creation Week described in Genesis. Whatever biological changes have occurred since Creation Week have accomplished only changes within the original created kinds.
3. The Great Flood described in Genesis, commonly referred to as the Noachian Flood, was a historical event worldwide in its extent and effect.
4. We are an organization of Christian men and women of science who accept Jesus Christ as our Lord and Savior. The act of the special creation of Adam and Eve as one man and woman and their subsequent fall into sin is the basis for our belief in the necessity of a Savior for all people. Therefore, salvation can come only through accepting Jesus Christ as our Savior.

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